INFORMATION TO USERS

This manuscript has been reproduced from the microfilm master. UMI films the text directly from the original or copy submitted. Thus, some thesis and dissertation copies are in typewriter face, while others may be from any type of computer printer.

The quality of this reproduction is dependent upon the quality of the copy submitted. Broken or indistinct print, colored or poor quality illustrations and photographs, print bleedthrough, substandard margins, and improper alignment can adversely affect reproduction.

In the unlikely event that the author did not send UMI a complete manuscript and there are missing pages, these will be noted. Also, if unauthorized copyright material had to be removed, a note will indicate the deletion.

Oversize materials (e.g., maps, drawings, charts) are reproduced by sectioning the original, beginning at the upper left-hand corner and continuing from left to right in equal sections with small overlaps.

ProQuest Information and Learning
300 North Zeeb Road, Ann Arbor, MI 48106-1346 USA
800-521-0600

UMI®

PHYLOGEOGRAPHY AND POPULATION GENETIC STRUCTURE OF BERINGIAN LANDBIRDS

A

DISSERTATION

Presented to the Faculty

of the University of Alaska Fairbanks

in Partial Fulfillment of the Requirements

for the Degree of

DOCTOR OF PHILOSOPHY

By

Christin Leigh Pruett, B.S., M.S.

Fairbanks, Alaska

December 2002

ProQuest Information and Learning Company
300 North Zeeb Road
P.O. Box 1346
Ann Arbor, MI 48106-1346

PHYLOGEOGRAPHY AND POPULATION GENETIC STRUCTURE OF BERINGIAN LANDBIRDS

By

Christin Leigh Pruett

RECOMMENDED:

Kent Schwarzyuk

Gary Dewton

THOMAS HAHN

Advisory Committee Chair

Edward C

Department Head, Biology and Wildlife

APPROVED:

D Woodall

Dean, College of Science, Engineering, and Mathematics

Dean of the Graduate School

11-11-02

Date

I. ABSTRACT

Molecular genetic approaches can be used to evaluate the historic and current relationships among populations. Mitochondrial DNA sequences and nuclear microsatellite loci were used to examine questions in avian community ecology, biogeography, and population genetics, including: 1) how have simple, high latitude bird communities been historically assembled; 2) how have past climate changes affected a species that has its entire distribution in an area that has experienced many glacial cycles; and 3) what are the genetic effects of sequential peripheral isolation in a natural vertebrate system?

Landbird communities in the Aleutian Islands are simple and replicated, having only eight members. Traditional community assembly theory would describe this co-distribution as being due to nonrandom, likely contemporary ecological factors. However, I found that many species had unique colonization and persistence patterns. Results suggest that these communities were assembled randomly, and that simple ecological assembly rules could not adequately describe this complex process.

Species endemic to Beringia, such as the rock sandpiper (*Calidris ptilocnemis*), would likely have been strongly affected by Pleistocene glacial cycles. MtDNA data suggest that these past climate changes have shaped current distribution and geographic variation in this species. Multiple instances of isolation and differentiation in glacial refugia and subsequent post-glacial population expansions are apparent. This study shows the complex biological responses of endemic Beringian species to climate change and isolation in glacial refugia.

Populations that are increasingly isolated from a species' main distribution should provide a useful model for examining the genetic effects of sequential peripheral isolation. Song sparrows (*Melospiza melodia*) in Alaska have such a distribution, and the most distant populations have morphological and behavioral differences concordant with trends in peripheral or island populations in other species. I examined mtDNA sequences and nuclear microsatellite loci that evolve at different rates, and found that the combined processes of genetic drift, isolation, and divergent selection likely caused rapid morphological and behavioral changes in the most peripheral populations. Results of this study suggest that the examination of molecular markers that evolve at different rates can provide insight into the processes that lead to subspecies differentiation or the first steps in speciation.

II. TABLE OF CONTENTS

III. LIST OF FIGURES

IV. LIST OF TABLES

V. INTRODUCTION

Molecular genetic approaches have been used extensively in the field of evolutionary biology, in part because these techniques enable researchers to infer the historic and current relationships of populations and species (Avise 1994). Recently, these techniques have been applied to questions previously thought to be difficult or impossible to answer using traditional techniques in a variety of biological disciplines (Avise 1994, 2000, Harvey et al. 1996, Landweber and Dobson 1999). My dissertation focuses on answering some of these questions in the fields of community ecology, biogeography, and population genetics using as models landbird populations that are distributed in Beringia: Alaska, eastern Siberia, and the Bering Strait.

Several factors make studying the genetic relationships of landbirds in Beringia of interest, including the history of major climate change in this region, which shaped the distributions of many organisms (Hopkins 1967, Hamilton et al. 1986, Pielou 1991); the location of a 1,800 km long east-to-west archipelago (Aleutian Islands) that connects two continents; and the relatively high incidence of endemic species and subspecies of birds distributed in this high-latitude region (Fay and Cade 1959, Gibson and Kessel 1997). I used two molecular data sets to examine landbird populations: mitochondrial DNA (mtDNA) sequences from two genes and nuclear microsatellite loci. MtDNA is maternally and clonally inherited and represents a direct line of descent with no recombination, while microsatellites are paternally and maternally inherited (Hillis et al. 1996). Also, these two markers evolve at different rates, with mtDNA being more useful for elucidating deeper historic events and microsatellites for recent and current processes

(Hillis et al. 1996). Through the use of two markers, I was able to examine both past and recent processes shaping populations of landbirds in Beringia.

In Chapter 1, I examine the history of colonization of the very simple eight-member landbird communities co-distributed in the Aleutian Islands of Alaska. I sought to determine whether simple ecological community assembly rules could explain community composition.

In Chapter 2, I examined the biogeographic history of the rock sandpiper (*Calidris ptilocnemis*), a Beringian endemic bird. I sought to determine the importance of climate change and glacial refugia on the establishment and differentiation of a species that has its entire breeding distribution in an area known for past glaciations.

In Chapter 3, I examined the genetic effects of varying degrees of geographic isolation on populations of song sparrows (*Melospiza melodia*) in Alaska. I sought to determine how historic and current processes had shaped sequentially peripheral populations in a natural vertebrate system.

I performed all DNA sequencing, genotyping, and analysis of data. My co-author on all three chapters, Dr. Kevin Winker, is my thesis advisor and provided assistance in project design, logistical and financial support, and editorial comments.

REFERENCES

Avise, J. C. 1994. Molecular markers, natural history, and evolution. Chapman and Hall, New York.

Avise, J. C. 2000. Phylogeography: The history and formation of species. Harvard University Press, Cambridge, Massachusetts.

Fay, F. H., and T. J. Cade. 1959. An ecological analysis of the avifauna of St. Lawrence Island, Alaska. University of California Publications in Zoology 63:73-150.

Gibson, D. D., and B. Kessel. 1997. Inventory of the species and subspecies of Alaska birds. Western Birds 28:45-95.

Hamilton, T. D., K. M. Reed, and R. M. Thorsen. 1986. Glaciation in Alaska. Alaska Geological Society, Anchorage, Alaska.

Harvey, P. H., A. J. L. Brown, J. M. Smith, S. Nee. 1996. New uses for new phylogenies. Oxford University Press, Oxford, U. K.

Hillis, D. M., C. Moritz, and B. K. Mable. 1996. Molecular systematics. Sinauer, Sunderland, Massachusetts.

Hopkins, D. M. 1967. The Bering land bridge. Stanford University Press, Stanford, California.

Landweber, L. F., and A. P. Dobson. 1999. Genetics and the extinction of species. Princeton University Press, Princeton, New Jersey.

VI. Chapter 1

Simple landbird communities defy simple assembly rules[1]

ABSTRACT

The role of history in community assembly has been hotly debated for the last 25 years. Traditionally, community ecologists have emphasized contemporary ecological processes in community assembly while evolutionary ecologists have focused on historic factors. I examined simple, replicated communities of breeding landbirds in the Aleutian Islands to determine whether simple assembly rules could explain community composition. Geologic evidence suggests that the Aleutian Islands were completely glaciated during the last glacial maximum, and thus these eight species probably colonized the Aleutian Islands within the last 10,000 – 12,000 years. Sequence data from the mtDNA cytochrome b gene (963 – 1,137 bp) from 318 individuals of eight species from two island communities and several possible source populations were examined. Six different genetic patterns of inferred colonization of these two replicated communities were found among the eight species. This finding is not significantly different from randomly assembling these two island communities. These results show that random, species-specific historic factors played a definitive role in Aleutian landbird community assembly in that identical end points have been achieved through different paths. The fact that these communities have replicated membership is essentially an accident of the present, rather than the duplication of nonrandom processes. The

[1] Pruett, C. L., and K. Winker. (in preparation). Simple landbird communities defy simple assembly rules. Evolution.

importance of random events in the shaping of such simple communities and the seemingly impossible task of defining an assembly rule for Aleutian landbirds raises the question of whether these rules exist for any community.

INTRODUCTION

The role of history in community assembly has confounded community ecologists for more than 25 years (Ricklefs and Schluter 1993; Weiher and Keddy 1999). The complexity of natural systems and a lack of analytical tools to assess history's role in community assembly led most early theorists to largely ignore the colonization and extinction histories of species found in communities (Wiens 1989; Losos 1996). Beginning with Diamond's (1975) seminal work on community assembly rules, many ecologists concluded that contemporary ecological processes, such as interspecific competition, were the most important determinants in species assemblages. These studies primarily examined the presence or absence of species in drawing conclusions about how and why species were distributed in particular areas (Diamond 1975; M'Closkey 1978; Fox and Brown 1993). An implicit assumption of this approach is that different islands or isolated patches represent replicated communities, and that the individual histories of species that are co-distributed in these replicated communities are of little importance to the present or future processes of assembly (Cody 1975; Simberlof et al. 1999).

With the advent of molecular techniques, evolutionary ecologists have begun challenging these basic assumptions by examining how speciation and dispersal affect community assembly. They argue that regional or historical processes can be as

important as local or contemporary processes in shaping communities (Ricklefs 1987; Losos 1996; Price et al. 2000; Ricklefs and Bermingham 2001). To further evaluate the importance of history, Drake (1990, 1991) used experimental manipulations of very simple, controlled microbial, algal, and invertebrate communities and concluded that the colonization history of species can have a dramatic impact on the assembly trajectory of communities. However, these findings have not been tested in an uncontrolled system. Recently, ecologists have examined simple natural communities in the hope of limiting the possible confounding effects of history (Booth and Larson 1999). However, it is unclear whether simple, naturally occurring communities are useful systems for examining community assembly and formulating assembly rules because the colonization histories of these communities have not been examined.

In this study, evidence was sought for assembly rules among simple, replicated landbird communities in the Aleutian Archipelago of Alaska (Fig 1.1). To do this, mitochondrial DNA sequence data were used to examine the colonization histories of the co-distributed landbird species found in the Aleutian Islands. Several features make these island communities an ideal natural experiment for determining assembly rules for simple, replicated, vertebrate communities. Eight species of landbirds breed throughout the 1800 km archipelago, including the rock ptarmigan (*Lagopus mutus*), rock sandpiper (*Calidris ptilocnemis*), common raven (*Corvus corax*), winter wren (*Troglodytes troglodytes*), song sparrow (*Melospiza melodia*), Lapland longspur (*Calcarius lapponicus*), snow bunting (*Plectrophenax nivalis*), and gray-crowned rosy-finch (*Leucosticte tephrocotis*). These eight species are not close relatives. Although three are

found in the same family (Emberizidae: song sparrow, Lapland longspur, and snow bunting), none are in the same genus, effectively minimizing the potential effects of interspecific competition in the assembly of these communities. Seven of the eight species are year-round residents in the Aleutians (Murie 1959; Byrd et al. 1974; Gibson 1981), making it likely that genetic data can recover past colonization history. One species is an annual migrant through the length of the archipelago (Lapland longspur) and serves as a natural control because it effectively recolonizes the Aleutians every year. This very simple eight-member avian community is replicated throughout the Aleutians, with few other landbird species breeding intermittently on some islands (Murie 1959).

In addition to having a very simple avian community structure, geological evidence suggests that the Aleutian Islands were completely glaciated during the last glacial maximum, being unavailable for colonization until 10,000 - 12,000 ybp (Barnosky et al 1987; Black 1983; Mann and Peteet 1994). Thus, Aleutian landbird communities appear to be an ideal case in which a shared colonization history should allow classic assembly rules (which ignore history) to emerge. By examining each species independently and then comparing their inferred colonization histories, I tested three key assumptions often invoked in community assembly theory 1) community assembly rules can be determined by examining the current distribution (presence or absence) of community members in replicated communities; 2) if communities have co-distributed species then they are replicates with the same assembly rules operating in each community, and 3) the colonization history of community members, especially in simple

communities, is unimportant and can be ignored when determining community assembly rules.

METHODS

Study area, sampling scheme, and molecular analyses

The Aleutian Islands are of volcanic origin and comprise one of the world's few island chains oriented in an east-west direction. The Aleutians have strong winds and cold temperatures throughout the year (Murie 1959), and habitats are simple (Hultén 1960). The two replicated Aleutian communities that were sampled, the Near Islands (52° 56'N 173° 15'E) and the Andreanof Islands (51° 45'N 176° 45'W), are found approximately 1800 km and 1000 km, respectively, from the Alaska mainland (Fig. 1.1).

Individuals of each of the eight species were sampled from the two locations in the Aleutian Islands (n = 10 per location) and from several possible source populations (n = 19-22; Fig. 1.1, Table 1.1). Whole genomic DNA from muscle tissue was extracted (Glenn 1997), and most of the mitochondrial cytochrome b gene (963 – 1137 bp) was amplified for each bird and cycle-sequenced using a variety of primer sets per individual. Amplified products were sequenced in both directions using an ABI 373A or 3100 automated sequencer (Applied Biosystems Inc.). Four primers were used for each species: Rock Ptarmigan (1, 2, 3, 4), Rock Sandpiper (3, 4, 5, 6), Common Raven (1, 3, 4, 6), Winter Wren (7, 8, 9, 10), Song Sparrow and Lapland Longspur (3, 4, 5, 6), Snow Bunting (1, 6, 11, 12), and Gray-crowned Rosy-Finch (1, 3, 4, 6), primer numbers correspond to the following: 1) L14841 (Helm-Bychowski and Cracraft 1993), 2) H16065 (Kocher et al. 1989), 3) L15424 (Hackett 1996), 4) L15350 (Klicka and Zink

1997), 5) L14851 (Kornegay et al. 1993), 6) H16064 (Harshman 1996), 7) L1650 (5' – ATTGCCTCCCAC CTAATCGACC – 3'), 8) H1150 (5' – GGAGGTTGGCAACTAGGGTTC – 3'), 9) L479 (5' – CCTAGTAGAGTGAGCGTGGGG – 3'), 10) H626 (5' – GGGTTGTTTGAACCTGTTTCG – 3'), 11) L519, and 12) H637 (Pruett et al. 2001). All sequences were deposited in Genbank (Table 1.1).

The same well-understood genetic marker, the mtDNA gene cytochrome b, with its relatively constant rate of evolution (Moore and DeFillipis 1997), was used to facilitate direct comparisons among species. Larger sample sizes (19-22 individuals) from several non-Aleutian populations in Alaska or Asia (Table 1.1, Fig. 1.1) were examined to increase the likelihood of detecting any putative Aleutian endemic haplotypes that might be found in possible source populations. Tentative dates of divergence were assigned to haplotypes by using the mean and 95% confidence interval (1.85% ± 0.79% per MY) of five avian cytochrome b molecular clocks developed independently for albatrosses (1.58% per MY and 2.86% per MY, Nunn et al. 1996), cranes (1.2% per MY, Krajewski and King 1996), partridges (2% per MY, Randi 1996), and Hawaiian honeycreepers (1.6% per MY, Fleischer et al. 1998).

Population structure and pattern-based analyses

For each species, three sampling sources were evaluated: the Near Islands, the Andreanof Islands, and a pooled area comprised of all sampled locations that could serve as possible sources for post-glacial colonization of the Aleutian Islands. Patterns of population structure within each species were then analyzed using a population pairwise

test for genetic differentiation (Arlequin, ver 1.1; Schneider et al. 1997). In this test, haplotype frequencies are compared between locations to determine whether significant differences occur. Species were then assigned to one of six patterns possible for relationships among three populations: 1) all three populations are significantly different; 2) the Near Islands are different from the other populations; 3) the Andreanof Islands are different from the other populations; 4) Aleutian Island populations are different from one another but not different from the possible source populations; 5) Aleutian Island populations are not different from each other but are different from possible source populations, and 6) all three locations are not different from one another.

The distribution of patterns among species was tested against a null model using a Monte Carlo method that simulated 10,000 random assemblies of these two eight-member communities. This test was used to determine whether the patterns discovered were significantly different from randomly assigning each species to one of the six possible patterns of colonization. To further examine relationships between island communities, population pairwise F_{st} estimates based on haplotype frequencies and genetic distances were determined (Arlequin, ver. 1.1; Schneider et al. 1997).

RESULTS

Post-glacial colonization

Given the moderate rate of evolution for cytochrome b in birds (~ 2% per MY; see references above), the evolution and fixation of new cytochrome b haplotypes in Aleutian populations is unlikely if colonization occurred after the last glacial maximum (<12,000 ybp). However, all species showed putative endemic Aleutian haplotypes

(Table 1.2; Fig. 1.2), haplotypes that were not found in possible source populations. Endemic haplotypes were 1 – 5 bp different (0.09 – 0.48% sequence divergence) from the genetically most similar haplotypes found in possible source populations. These levels of differentiation correspond to molecular divergence dates that precede the last glacial maximum (33,000 to 455,000 ybp; Table 1.2; Fig. 1.2). In addition, several species possess endemic haplotypes that are fixed within one or both Aleutian Island populations (Table 1.3). The Andreanof Island population of rock ptarmigan has one fixed endemic haplotype. All common ravens examined from the Near Islands have one of three endemic haplotypes. Both island populations of winter wrens and song sparrows have several (four and three, respectively) fixed Aleutian Island endemic haplotypes. Thus, some Aleutian populations have lineages that predate the last glacial maximum and have also become fixed.

Although several Aleutian lineages have molecular divergence dates that appear to predate a post-glacial colonization, another possible explanation is that small founder populations colonized the Aleutians post-glacially and through genetic drift new (Aleutian endemic) mutations were rapidly fixed. However, all seven resident species would have had to evolve at least one mutation within the last 10,000 – 12,000 yrs (Table 1.2). Given that cytochrome b evolves at approximately 2% per MY, there is approximately a 20% chance of a single mutation occurring in the sequenced portion of this gene within a 10,000 yr period. The probability of independent mutations (P) occurring across all species was calculated using the following formula:

$$P = x^y$$

where x is the probability of a single mutation arising in a single species (0.20) in a 10,000 year period and y is the number of resident species (7) with at least one endemic mutation. The probability of independent mutations occurring in this gene in 10,000 years across all species is $P = 0.000013$. Further, in a total of four populations these mutations would have had to go to fixation through drift (Table 1.3), another time consuming process assuming that these mutations are neutral.

Rather than a single colonization event of all species following the last glacial maximum, an alternative scenario for the variable patterns found in this study might be that the eight Aleutian landbird species were all isolated by the last glacial maximum in the same refugia. Subsequently, gene flow between Aleutian Island and other populations of Lapland longspurs, snow buntings, and gray-crowned rosy-finches might have obscured most of the refugial signal for these species. Although endemic haplotypes were found for Lapland longspurs, snow buntings, and gray-crowned rosy-finches, eight of the nine haplotypes are singlets (only one individual had the haplotype; Table 1.2), and none were shared between the two Aleutian populations. If these haplotypes were due to a refugial event (rather than a recent mutation), it is likely that they would be found in higher frequencies in Aleutian populations given the current limited movement patterns of snow buntings and gray-crowned rosy-finches (Murie 1959).

The low probability of seven independent mutations evolving within the last 10,000 yrs, the fact that in many cases these putative endemic haplotypes have become fixed, and the robust sampling scheme from multiple possible source populations (Fig.

1.1) strongly suggest that some Aleutian populations were isolated during the last glacial maximum. Thus, there is clearly a strong indication that some members of these communities colonized the Aleutian Islands before the last glacial maximum.

Shared colonization histories

If Aleutian landbird populations shared the same colonization history, all species should have similar haplotype frequency patterns. In addition, if Aleutian landbirds colonized post-glacially then all species should share the same haplotype frequency pattern as the Lapland longspur: there should be no significant haplotype frequency differences between island and possible source populations. As expected, Lapland longspur populations show no significant differences in haplotype frequencies (Table 1.3). Snow buntings and gray-crowned rosy-finches share this lack of differentiation among populations. In contrast, significant differences between Aleutian Island and possible source populations were found for rock ptarmigan, common ravens, winter wrens, and song sparrows, while Aleutian Island populations of rock sandpiper were different from one another but not from possible source populations (Table 1.3). No haplotypes were shared between populations that showed significant differentiation, except for Aleutian populations of winter wren. Near Island and Andreanof Island populations of winter wrens shared two haplotypes that were not found in possible source populations (Table 1.3). These population differences correspond to all six possible patterns (Fig. 1.3; also see Methods). Thus, each species appears to have an independent colonization history.

If both island communities had the same colonization history, then comparisons between islands for each species would show similar low F_{st} estimates of population divergence. However, F_{st} values varied widely among species (Fig. 1.2) with rock otarmigan showing the highest level of differentiation (1.00) and Lapland longspurs the lowest (<0.01). Thus, the histories of Andreanof and Near Island populations are strikingly different among species, suggesting fundamental differences in colonization history.

Non-random community assembly

If Aleutian landbird communities had the same colonization history, then a non-random assembly of these species would be likely. Simulations indicate that a significantly nonrandom assembly (i.e., species showing predominantly shared histories) would only be indicated if one or two colonization patterns had been found (Fig. 1.4). However, all six possible patterns occurred among the eight species (Fig. 1.3). Simulations indicate that finding six patterns among these species is not significantly different from randomly assigning each species a colonization history (Fig. 1.4). Thus, these Aleutian landbird communities were assembled through a random colonization process.

Complex colonization history

Little is known about Aleutian Island glacial history before the last glacial maximum (Black 1983), however, molecular clock estimates indicate that song sparrows (33,000 – 331,000 ybp), rock ptarmigan (37,000 – 91,000 ybp), and winter wrens (39,000 – 391,000 ybp) have similar molecular divergence dates that might be linked to an

interstadial or interglacial that occurred during the Pleistocene (Hamilton et al. 1986, Williams et al. 1998). A conservative estimate, based on the most recent divergence dates, indicates that these species were isolated 30,000 – 40,000 ybp which corresponds loosely to a recent interstadial event (approximately 25,000 – 35,000 ybp; Hamilton et al. 1998). While these species show similar divergence estimates, common ravens have older divergence dates (110,000 – 455,000 ybp) that might correspond to much older glacial events (Hamilton et al. 1998), which suggests that some raven populations have persisted in the Aleutian Islands for a longer period of time than other landbird species. These different divergence estimates indicate that there were probably three different colonization events for Aleutian landbird species: 1) an initial colonization by common ravens; 2) colonization by rock ptarmigan, song sparrows, and winter wrens possibly during a recent interstadial; and 3) a post-glacial colonization by Lapland longspurs, snow buntings, and gray-crowned rosy-finches.

DISCUSSION

Assembly rules

Evidence was sought for a simple ecological assembly rule to explain simple, replicated communities of landbirds in the Aleutian Islands. After examining the histories of each species in each community, formulation of a simple rule seems impossible. A complex mixture of habitat availability (ice free refugia), species availability (suitable colonists), and persistence (of refugial populations and habitats) appears to have driven the assembly of these Aleutian landbird communities. These

factors and the resultant processes that led to these replicated vertebrate communities cannot be distinguished from a purely random process of assembly (Fig. 1.4).

Species-specific histories

If the current presence or absence of species among communities was used as the primary factor in determining assembly rules, then incorrect conclusions about how and why certain species are currently found in the Aleutian Islands would be drawn. Although the eight species represent a very simple community, an examination of each species' history in the Aleutian Islands shows an amazing heterogeneity (Fig. 1.2). Both intra- and inter-specific variability in colonization histories and probable source populations are evident. Rock sandpipers provide a good example of differential colonizing sources for each island group. The Near and Andreanof island populations do not share any haplotypes (Table 1.3), but haplotypes from both locations are found in different possible source areas. These findings indicate that different source populations probably colonized each island.

Another unusual colonization pattern is found in rock ptarmigan. Although Andreanof Island ptarmigan are distinct from all other populations (see also Holder et al. 2000), the Near Island population is not different from birds found on the mainland of Alaska or Asia (Fig. 1.3). Thus, the Near Islands were probably recently colonized from Asia (the nearest undifferentiated source).

Near Island populations of common raven and Aleutian populations of winter wren and song sparrow further exemplify the complexity of species-specific histories.

Although these three species have endemic, fixed lineages that predate the last glacial maximum, the ages of these lineages are highly variable. Common ravens have the oldest lineages (110,000 – 455,000 ybp) that appear to have persisted through several glacial cycles, while winter wrens (39,000 – 391,000 ybp) and song sparrows (33,000 – 83,000 ybp) possess much younger lineages (Fig. 1.3).

Recent molecular studies of the phylogeography of rock ptarmigan (Holder et al. 1999; 2000), marbled murrelets (*Brachyramphus marmoratus*; Congdon et al. 2000), and pigeon guillemots (*Cepphus columba*; Kidd and Friesen 1998) also found evidence for Aleutian glacial refugia. Molecular clock estimates of isolation dates for Aleutian populations of marbled murrelets (50,000 ybp; Congdon et al. 2000) and rock ptarmigan (39,000 – 43,000 ybp; Holder et al. 2000) correspond well with estimates from this study for populations of song sparrows (33,000 – 248,000 ybp), winter wrens (39,000 – 293,000 ybp), and rock ptarmigan (37,000 – 91,000 ybp). It is important to note that Holder et al. (2000) and Congdon et al. (2000) used different molecular clocks (calibrated for nuclear introns and the mitochondrial control region) and obtained isolation dates that tend to agree with the cytochrome b estimates presented here. These independent studies show that several Aleutian populations likely persisted in refugia through the last glacial maximum.

Islands as replicated communities

In this study considerable heterogeneity in estimated divergence dates, ages of endemic lineages, and the persistence of these lineages between the Andreanof and Near Island communities is evident (Fig. 1.2). In addition, pairwise F_{st} estimates between

these Aleutian communities show a remarkable range of values (Fig. 1.2). Thus each island community has a unique history, and although both islands share the same species they were not formed by the same colonization and persistence histories. The assumption that when islands share the same species, they can be treated as replicates is not always valid because it is just as likely that very different processes and factors shaped each community independently.

Importance of history

One might argue that even though species colonized at different times and from different source populations, what really matters is that these eight species are currently co-distributed and essentially constitute replicate communities. However, an examination of the colonization histories of rock ptarmigan, common ravens, winter wrens, and song sparrows shows how species-specific historical events that do not differ from random processes among species can profoundly shape current distributional patterns. Rock ptarmigan have very limited movement patterns throughout their Holarctic breeding range and are non-migratory in the Aleutian Islands (Murie 1959, Holder and Montgomerie 1993). Based on both control region (Holder et al. 2000) and cytochrome b estimates (Table 1.2), the Andreanof Island population of rock ptarmigan probably has not received colonists for approximately 30,000 years. A logical conclusion from these results is that rock ptarmigan would not currently be found in the Andreanof Islands if not for a pre-glacial colonization event, because they would not have had the ability or time to colonize after the last glacial maximum.

Some non-Aleutian populations of song sparrows and winter wrens are migratory, there are fixed Aleutian endemic haplotypes for each of these species. Molecular clock estimates show that the Aleutian populations of winter wrens and song sparrows have likely not received colonists for at least 30,000 years. Whatever the processes (weather, remote island locations) that may have limited the movement capabilities of these species into the Aleutian Islands after the last glacial maximum, it is likely that the current community structure would be radically different if not for the persistence of these species in glacial refugia.

A simple ecological assembly rule based on habitat suitability would not adequately explain why rock ptarmigan, common ravens, winter wrens, and song sparrows are currently distributed throughout the Aleutian Archipelago. Such an assembly rule would ignore one of the most striking conclusions from this study: the currently co-distributed landbird communities are an accident of the present. The data strongly suggest that at different times in history these islands could have had very different community compositions.

Conclusions

Simple, replicated landbird communities that share similar climate, habitat diversity, and very low or nonexistent interspecific competition provide an ideal situation in which to find true ecological assembly rules. An examination of the genetic (and thus inferred colonization) histories of these communities shows that intraspecific, interspecific, and inter-island differences are remarkably complex. The important role

history has played in shaping such simple replicated communities suggests that simple assembly rules might not exist in any system.

ACKNOWLEDGMENTS

This project was supported by the Angus Gavin Memorial Bird Research Grant, a National Science Foundation EPSCoR genomics fellowship, the University of Alaska Museum, the National Geographic Society, the U.S. Department of Agriculture, and an anonymous donor. Field and technical support were provided by the U.S. Fish and Wildlife Service, U.S. Coast Guard, and U.S. Air Force. I thank the personnel at the Kodiak, Izembek, Togiak, and Alaska Maritime National Wildlife Refuges, Bureau of Land Management, and the Alaska Department of Fish and Game - Nome for allowing use of vehicles and facilities. I thank C. M. Topp for landbird silhouettes. I also thank K. Winker, D. D. Gibson, G. M. Spellman, J. J. Weicker, R. W. Dickerman, M. C. Thompson, T. M. Boucher, D. A. Rocque, A. B. Johnson, K. G. McCracken, and T. M. Braile for technical assistance and field sampling. Winter wren, rock otarmigan, and common raven tissues from Asia were provided by Sievert Rowher of the Burke Museum, University of Washington. For helpful comments and discussion, I thank A. Denton, T. Hahn, K. Schwaegerle, and K. Winker.

REFERENCES

Barnosky, C. W., P. M. Anderson, and P. J. Bartlein. 1987. The northwestern U. S. during deglaciation: Vegetational history and paleoclimactic implications. Pp. 289-321 *in* W. F. Ruddiman and H. E. Wright, Jr. eds. North America and

adjacent oceans during the last deglaciation. Geological Society of America, Boulder, Colorado.

Black, R. F. 1983. Glacial chronology of the Aleutian Islands. Pp. 5-10 *in* R. M. Thorson and T. D. Hamilton, eds. Glaciation in Alaska. Alaskan Quaternary Center, University of Alaska Museum, Occasional Paper No. 2, Fairbanks, Alaska.

Booth, B. D., and D. W. Larson. 1999. Impact of language, history, and choice of system on the study of assembly rules. Pp. 206-229 *in* E. Weiher and P. Keddy, eds. Biological assembly rules. Cambridge University Press, Cambridge, UK.

Byrd, G. V., D. D. Gibson, and D. L. Johnson. 1974. The birds of Adak Island, Alaska. Condor 76:288-300.

Cody, M. L. 1975. Toward a theory of continental species diversities: Bird distributions over Mediterranean habitat gradients. Pp. 214-257 *in* M. L. Cody and J. M Diamond, eds. Ecology and evolution of communities. Harvard University Press, Cambridge, Massachusetts.

Congdon, B. C., J. F. Piatt, K. Martin, and V. L. Friesen. 2000. Mechanisms of population differentiation in marbled murrelets: Historical versus contemporary processes. Evolution 54:974-986.

Diamond, J. M. 1975. Assembly of species communities. Pp. 342-444 *in* M. L. Cody and J. M. Diamond eds. Ecology and evolution of communities. Harvard University Press, Cambridge, Massachusetts.

Drake, J. A. 1990. Communities as assembled structures: do rules govern pattern? Trends in Ecology and Evolution 5:159-164.

Drake, J. A. 1991. Community-assembly mechanics and the structure of an experimental species ensemble. American Naturalist 137:1-26.

Fleischer, R. C., C. E. McIntosh, and C. L. Tarr. 1998. Evolution on a volcanic conveyor belt: using phylogeographic reconstructions and K-Ar-based ages of the Hawaiian Islands to estimate molecular evolutionary rates. Molecular Ecology 7:533-545.

Fox, B. J., and J. H. Brown. 1993. Assembly rules for functional groups in North American desert rodent communities. Oikos 67:358-370.

Gibson, D. D. 1981. Migrant birds at Shemya Island, Aleutian Islands, Alaska. Condor 83:65-77.

Glenn, T. 1997. Genetic bottlenecks in long-lived vertebrates: Mitochondrial and microsatellite DNA variation in American alligators and whooping cranes. Ph. D. dissertation, University of Maryland, College Park.

Hackett, S. J. 1996. Molecular phylogenetics and biogeography of tanagers in the genus *Ramphocelus* (Aves). Molecular Phylogenetics and Evolution 5:368-382.

Hamilton, T. D., K. M. Reed, and R. M. Thorsen. 1986. Glaciation in Alaska. Alaska Geological Society, Anchorage, Alaska.

Harshman, J. 1996. Phylogeny, evolutionary rates, and ducks. Ph. D. dissertation, Univ. of Chicago, Chicago, Illinois.

Helm-Bychowski, K., and J. Cracraft. 1993. Recovering phylogenetic signal from DNA sequences: Relationships within the corvine assemblage (class Aves) as inferred from complete sequences of the mitochondrial DNA cytochrome-b gene. Molecular Biology and Evolution 10:1196-1214.

Holder, K., and R. Montgomerie. 1993. Rock ptarmigan (*Lagopus mutus*). Pp. 1-24 *in* A. Poole and F. Gill eds. The birds of North America, No. 51. The American Ornithologists' Union, Philadelphia, PA and Academy of Natural Sciences, Washington, D. C.

Holder, K., R. Montgomerie, and V. L. Friesen. 1999. A test of the glacial refugium hypothesis using patterns of mitochondrial and nuclear DNA sequence variation in rock ptarmigan (*Lagopus mutus*). Evolution 53: 1936-1950.

Holder, K. R., R. Montgomerie, and V. L. Friesen. 2000. Glacial vicariance and historical biogeography of rock ptarmigan (*Lagopus mutus*) in the Bering region. Molecular Ecology 9:1265-1278.

Hultén, E. 1960. Flora of the Aleutian Islands, 2nd ed. J. Cramer, New York.

Kidd, M. G., and V. L. Friesen. 1998. Analysis of mechanisms of microevolutionary change in *Cepphus* guillemots using patterns of control region variation. Evolution 52:1158-1168.

Klicka, J., and R. M. Zink. 1997. The importance of recent ice ages in speciation: a failed paradigm. Science 277: 1666-1669.

Kocher, T. D., W. K. Thomas, A. Meyer, S. V. Edwards, S. Pääbo, F. X. Villablanca, and A. C. Wilson. 1989. Dynamics of mitochondrial DNA evolution in animals:

amplification and sequencing with conserved primers. Proceedings of the National Academy of Science USA 86:6196-6200.

Kornegay, J. R., Kocher, T. D., Williams, L. A., and Wilson, A. C. 1993. Pathways of lysozyme evolution inferred from the sequences of cytochrome b in birds. Journal of Molecular Evolution 37:367-379.

Krajewski, C., and D. G. King. 1996. Molecular divergence and phylogeny: Rates and patterns of cytochrome b evolution in cranes. Molecular Biology and Evolution 13:21-30.

Losos, J. B. 1996. Phylogenetic perspectives on community ecology. Ecology 77:1344-1354.

Mann, D. H., and D. M. Peteet. 1994. Extent and timing of the last glacial maximum in southwestern Alaska. Quaternary Research 42:136-148.

M'Closkey, R. T. 1978. Niche separation and assembly in four species of Sonoran desert rodents. American Naturalist 112:683-694.

Moore, W. W., and V. R. DeFilippis. 1997. The window of taxonomic resolution for phylogenies based on mitochondrial cytochrome b. Pp. 84-115 *in* D. P. Mindell, ed. Avian molecular evolution and systematics. Academic Press, San Diego, California.

Murie, O. J. 1959. Fauna of the Aleutian Islands and Alaska Peninsula. North American Fauna No. 61, U. S. Fish and Wildlife Service, Washington D. C.

Nunn, G. B., J. Cooper, P. Jouventin, C. J. R. Robertson, and G. G. Robertson. 1996. Evolutionary relationships among extant albatrosses (Procellariiformes:

Diomedeidae) established from complete cytochrome b gene sequences. Auk 113:784-801.

Price, T., I. J. Lovette, E. Bermingham, H. L. Gibbs, and A. D. Richman. 2000. The imprint of history on communities of North American and Asian warblers. American Naturalist 156:354-367.

Pruett, C. L, D. D. Gibson, and K. Winker. 2001. Molecular "cuckoo clock" suggests listing of western yellow-billed cuckoos may be warranted. Wilson Bulletin 113:228-231.

Randi, E. 1996. A mitochondrial cytochrome b phylogeny of the *Alectoris* partridges. Molecular Phylogenetics and Evolution 6:214-227.

Ricklefs, R. E. 1987. Community diversity: Relative roles of local and regional processes. Science 235:167-171.

Ricklefs, R. E., and D. Schluter. 1993. Species diversity in ecological communities. University of Chicago Press, Illinois.

Ricklefs, R. E., and E. Bermingham. 2001. Nonequilibrium diversity dynamics of the Lesser Antillean avifauna. Science 294:1522-1524.

Schneider, S., J. Kueffer, D. Roessli, and L. Excoffier. 1997. Arlequin ver. 1.1: A software for population genetic data analysis. Genetics and Biometry Laboratory, University of Geneva, Switzerland. http://lgb.unige.ch/arlequin/.

Simberlof, D., L. Stone, and T. Dayan. 1999. Ruling out a community assembly rule: the method of favored states. Pp. 58-74 *in* E. Weiher and P. Keddy, eds.

Biological assembly rules, perspectives, advances, retreats. Cambridge University Press, Cambridge, UK.

Weiher, E., and P. Keddy. 1999. Biological assembly rules: Perspectives, advances, retreats. Cambridge University Press, Cambridge, UK.

Weins, J. A. 1989. The ecology of bird communities. Vol 1. Foundations and patterns. Cambridge University Press, Cambridge, UK.

Williams, M., D. Dunkerley, P. De Deckker, P. Kershaw, and J. Chappell. 1998. Quaternary Environments, 2nd ed. Arnold, London, U. K.

Table 1.1. Species, sample locations, sample sizes, and Genbank accession numbers for cytochrome b sequences used in this study. Detailed information on locations, collection dates, and museum specimen voucher numbers are provided on Genbank.

Species	Near Is. (n)	Andreanof Is. (n)	Source[a]
Rock Ptarmigan	10	10	1(9), 2(11), 3(2)
Genbank accession	AY156305-314	AY156315-324	AY156325-346
Rock Sandpiper	10	10	1(9), 2(2), 4(9)
Genbank accession	AY156223-232	AY156233-242	AY156243-262
Common Raven	10	10	1(5), 3(2), 5(15)
Genbank accession	AY156263-272	AY156273-282	AY156283-304
Winter Wren	10	10	3(2), 6(6), 7(1), 8(11), 9(2)
Genbank accession	AY156466-475	AY156476-485	AY156486-507
Song Sparrow	10	10	1(6), 7(10), 8(4)
Genbank accession	AY156386-395	AY156396-405	AY156406-425
Lapland Longspur	10	10	1(10), 2(1), 4(6), 5(2), 10(1)
Genbank accession	AY156183-192	AY156193-202	AY156203-222
Snow Bunting	10	10	1(10), 2(1), 4(5), 11(4)
Genbank accession	AY156426-435	AY156436-445	AY156446-465
Gray-crowned Rosy-Finch	10	10	1(1), 4(2), 12(3), 13(13)
Genbank accession	AY156347-356	AY156357-366	AY156367-385

[a] Possible source populations as numbered in Fig. 1, with sample sizes in parenthesis.

Table 1.2. Number and distribution of endemic Aleutian haplotypes for each species, percentage of individuals in each population possessing Aleutian endemic haplotypes (% Ind.), percent sequence divergence from most similar haplotype from possible source populations (% Div.), and range of tentative divergence dates (YBP) based on an avian molecular clock for cytochrome b (1.85% ± 0.79% per MY; see references in text).

	Near Islands				Andreanof Islands			
Species	n	% Ind.	% Div.	YBP	n	% Ind	% Div.	YBP
Rock Ptarmigan	0	0	-----	-----	2	100	0.10	36,760 – 91,102
Rock Sandpiper	2	20	0.10 – 0.19	36,904 – 182,913	4	40	0.10 – 0.19	36,904 – 182,913
Common Raven	3	90	0.29 - 0.48	110,069 – 454,632	0	0	-----	-----
Winter Wren	2	100	0.10 - 0.21	39,433 – 195,449	3	100	0.10 – 0.42	39,433 – 390,898
Song Sparrow	3	100	0.09 – 0.35	33,398 – 331,078	1	100	0.09	33,398 – 82,769
Lapland Longspur	0	0	-----	-----	3	38	0.09 – 0.18	33,457 – 165,830
Snow Bunting	2	20	0.09	33,814 – 83,801	3	30	0.09	33,814 – 83,801
Gray-crowned Rosy-Finch	1	22	0.10	37,048 – 91,813	0	0	-----	-----

Table 1.3. Number of cytochrome b base pairs (bp) examined, percentage of haplotypes shared between locations (% haps), and P-values from pairwise population differentiation tests between Near Islands (NI), Andreanof Islands (AI), and possible source populations (SP) for each Aleutian landbird species.

Species	bp	AI-SP	% haps	NI-SP	% haps	NI-AI	% haps
Rock Ptarmigan	1033	<0.01	0	0.18	56	<0.01	0
Rock Sandpiper	1029	0.23	53	0.08	43	0.03	0
Common Raven	1035	0.15	50	<0.01	0	<0.01	0
Winter Wren	963	<0.01	0	<0.01	0	0.04	70
Song Sparrow	1137	<0.01	0	<0.01	0	0.47	90
Lapland Longspur	1135	0.63	57	0.85	73	0.83	80
Snow Bunting	1123	0.56	63	0.31	67	0.73	75
Gray-crowned Rosy-Finch	1025	0.74	83	0.28	79	0.10	75

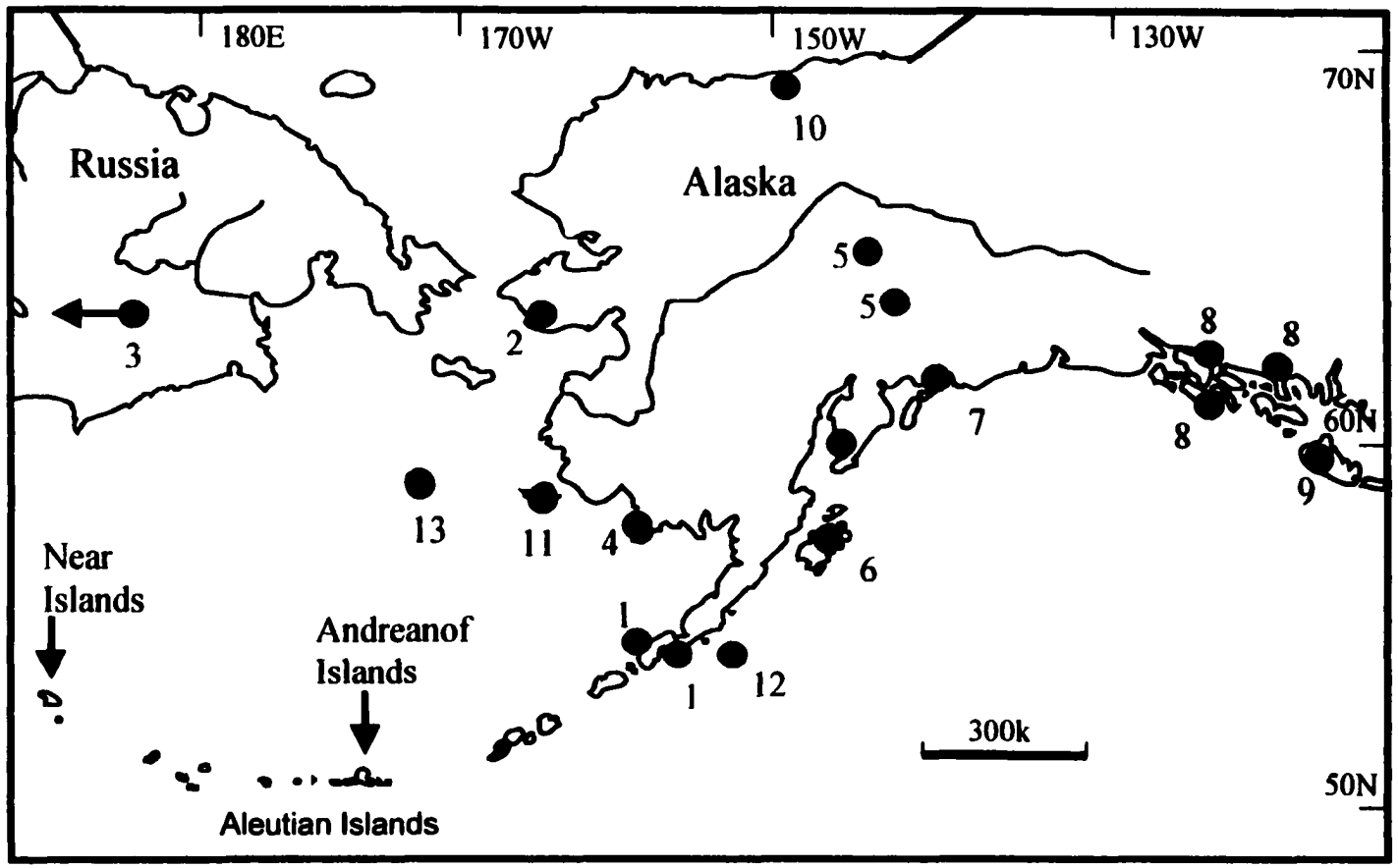

Fig. 1.1. The Aleutian Islands, Alaska mainland, and a portion of eastern Russia. Black circles represent sample locations among possible source populations. Arrows point to the two replicated island communities sampled in the Aleutian Islands and to Asian collection locations. Numbered locations are 1) Alaska Peninsula, 2) Seward Peninsula, 3) Asia, 4) Cape Peirce, 5) interior Alaska, 6) Kodiak Island, 7) Copper River Delta, 8) southeast Alaska, 9) Queen Charlotte Islands, 10) northern coastal Alaska, 11) Nunivak Island, 12) Shumagin Islands, and 13) Pribilof Islands.

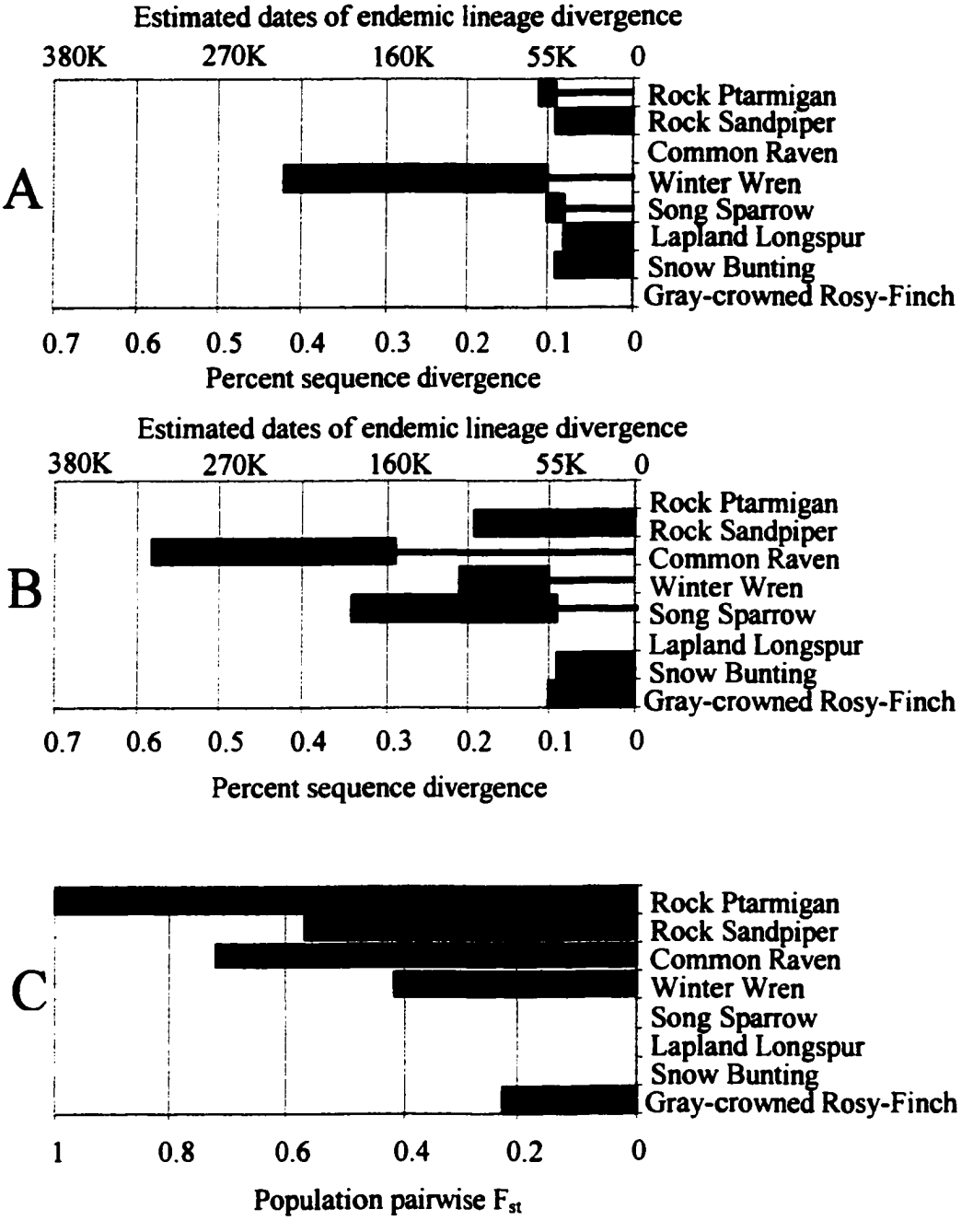

Fig. 1.2. Estimated dates of endemic lineage divergence and lineage persistence based on avian cytochrome b molecular clock (see Methods) and percent sequence divergence between endemic and closest possible source population haplotypes for the eight Aleutian community members for A) Andreanof Island and B) Near Island populations. Thick black lines represent estimated dates of endemic divergence; thin lines show inferred island persistence of these endemic haplotypes. Populations pairwise Fst values C) between Andreanof and Near Island populations of each of the eight Aleutian landbird species.

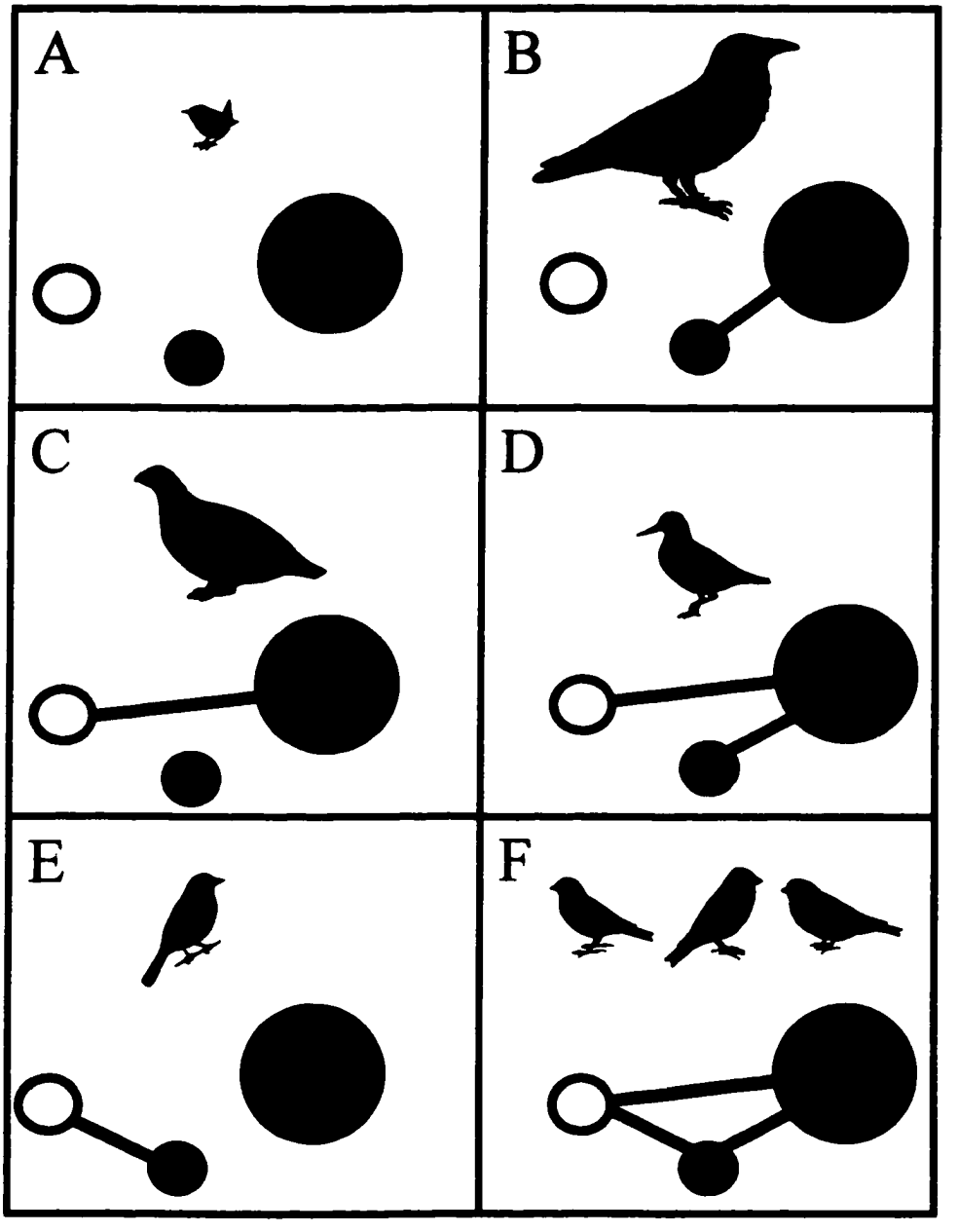

Fig. 1.3. Six possible patterns of the relationships among two island populations and a grouped possible source population with silhouettes of each species that showed the pattern. Large gray circles correspond to possible source populations, black circles to Andreanof Island populations, and white circles to Near Island populations. Lines connecting circles indicate a lack of significant genetic difference ($P > 0.05$) between locations. Patterns exhibited by the data were Winter Wren, *Troglodytes troglodytes* (A), Common Raven, *Corvus corax* (B), Rock Ptarmigan, *Lagopus mutus* (C), Rock Sandpiper, *Calidris ptilocnemis* (D), Song Sparrow, *Melospiza melodia* (E), Snow Bunting, *Plectrophenax nivalis*, Gray-crowned Rosy-finch, *Leucosticte tephrocotis*, and Lapland Longspur, *Calcarius lapponicus* (F).

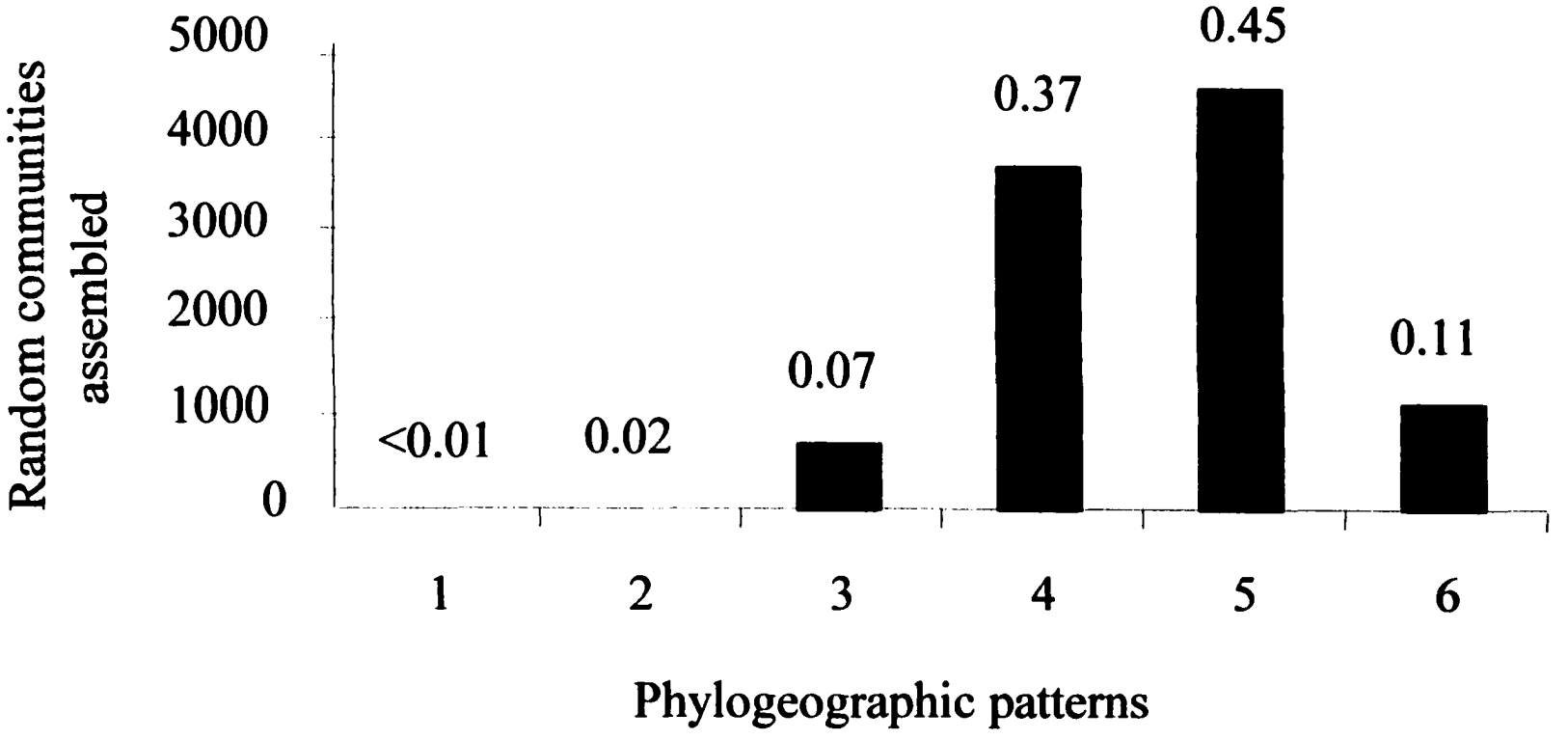

Fig. 1.4. Histogram showing results of Monte Carlo simulation of randomly assembling two communities of eight members from a source location. In this simulation, the eight species are randomly assigned to one of six assembly patterns (all patterns possible given that there are two island communities and a source population) and then this process is replicated 10,000 times. Numbers above bars represent P-values for the exact frequency of occurrence of one, two, three, four, five, or six different patterns for 10,000 replicated eight-member communities.

VII. Chapter 2

Establishment and differentiation of an endemic Beringian bird: the rock sandpiper[2]

ABSTRACT

The relative importance of climate change and refugia in the establishment and differentiation of species that are endemic to Beringia is largely unknown. The rock sandpiper (*Calidris ptilocnemis*) is a good test case for examining the biological effects of historic climate change in this region because it has a known sister species, the purple sandpiper (*Calidris maritima*) and is the only endemic Beringian bird with multiple described subspecies, suggesting a process of initial establishment and secondary differentiation through time in Beringia. I sequenced 2,074 bp of mtDNA from 40 rock sandpipers from nine breeding locations and four purple sandpipers. I then used maximum likelihood and Bayesian analyses to construct phylogenetic trees and pairwise coalescence methods to evaluate population demography, migration rates, and times of population divergence. Both phylogenetic trees have identical topologies and show that purple and rock sandpipers are sister species that diverged 1.5 Mya, with each species forming a monophyletic clade. Within the rock sandpiper clade branches are short but show population structure that roughly corresponds to some subspecies differences and indicate that rock sandpipers colonized North America from Asia. Coalescence estimates suggest that there were multiple refugial populations that correspond loosely to different glacial cycles. These findings suggest that climate change over the past 1.5 MY has

---

[2] Pruett, C. L., and K. Winker. (in preparation). Establishment and differentiation of an endemic Beringian bird: the rock sandpiper. Molecular Ecology.

strongly affected the distribution and differentiation of rock sandpipers. This study illustrates the important roles that climate change and refugia played in the evolution of an endemic Beringian species.

INTRODUCTION

Beringia, which includes most of Alaska, northeastern Siberia, and the Bering Strait, was strongly affected by climate change during the Wisconsin glaciation (10,000 to 117,000 ybp; Hamilton et al. 1986, Williams et al. 1998). Geologic evidence suggests that during this hundred-thousand year glacial period there were multiple cycles of warming and cooling (interstadials) which likely caused the expansion and contraction of ice-free habitats (Hopkins et al. 1982, Hamilton et al. 1986). These glacial cycles also caused sea levels to fluctuate, which intermittently exposed and submerged the Bering land bridge that connected Asia and North America (Hopkins 1967, Hopkins et al. 1982, Pielou 1991; Fig. 2.1).

By the time of the last glacial maximum (LGM: 10,000 to 30,000 ybp), ice covered substantial portions of the current distributions of birds breeding in Beringia. However, ice-free areas probably served as refugia for many species (Hamilton et al. 1986, Hewitt 2000; Stewart and Lister 2001; Fig. 2.1). There is evidence that at least one large glacial refugium was present in Beringia in the areas north of the ice in the Bering land bridge area and in present-day Alaska and Siberia (Pielou 1991; Fig. 2.1). Researchers studying the phylogeography of Rock Ptarmigan (*Lagopus mutus*; Holder et al. 1999) and Marbled Murrelets (*Brachyramphus marmoratus*; Congdon et al. 2000) have postulated that one or two previously unknown refugia were present in the Bering

Sea region, likely in the Aleutian Islands. However, there is currently a lack of geological and fossil evidence to suggest whether these Bering Sea refugia were present.

Species that have all or most of their breeding distributions in Beringia would have been strongly affected by these climate changes, and thus could serve as useful models for examining the biological effects of climate change and the importance of refugia during the Wisconsin period. I examined the phylogeographic and demographic history of the rock sandpiper (*Calidris ptilocnemis*) a species endemic to Beringia.

Several factors make rock sandpipers ideal for determining how climate change has affected Beringia. First, because both morphological and molecular evidence has shown that purple sandpipers are the sister taxon of rock sandpipers (Borowik and McLennan 1999), it is possible to examine whether the Wisconsin glacial cycles caused recent speciation between these two taxa. These two species are very similar morphologically, and at one time were considered to be the same species ("*Arquatella*" *maritima*; AOU 1910). Later they were separated because of differences in breeding plumage and disjunct breeding distributions (AOU 1931). Although current gene flow is unlikely, at least one purple sandpiper has occurred in Alaska in the last 20 years (Gibson and Kessel 1992). Even though these populations currently have different breeding ranges, it is possible that rock sandpipers diverged from purple sandpipers during the Wisconsin glaciation (< 117,000 ybp).

Second, rock sandpipers are the only Beringian endemic with described subspecies (AOU 1957), having four: *ptilocnemis*, *tschuktschorum*, *couesi*, and *quarta* (Peters 1934, AOU 1957, Gibson and Kessel 1997; Fig. 2.2). Other endemic birds in

Beringia are thought to be monotypic (AOU 1957, Fay and Cade 1959), and thus fit a model of a single Beringian refugium. Subspecies level differentiation in rock sandpipers suggests the possibility of multiple glacial refugia in this region. However, intergrades between subspecies have been documented (Murie 1959), and the distributional limits of some subspecies are uncertain. It is unclear whether these subspecific differences are due to protracted isolation in several glacial refugia, or to variable ecological factors affecting different populations, or to both. By examining each subspecies, it is possible to determine whether a single refugium or multiple refugia harbored rock sandpipers in the Wisconsin period and determine how past episodes of climate change have affected an endemic Beringian species.

Third, a portion of the current breeding distribution of rock sandpipers was glaciated during the LGM (Figs. 2.1, 2.2), and thus a scenario of postglacial population expansion out of glacial refugia is likely. Several unglaciated areas might have served as sources for population expansions, including a large Beringian refugium or a Bering Sea refugium. Most subspecies distributions are near at least one of these possible refugia (Figs. 2.1, 2.2). Thus, an examination of the phylogeography of rock sandpipers throughout much of their breeding distribution should provide insight into whether the currently recognized subspecific differences are due to isolation in different glacial refugia.

I sequenced two portions of the mitochondrial genome from all of the subspecies ranges of rock sandpipers and from several purple sandpipers and examined the phylogeographic patterns of these taxa. I sought to evaluate several questions about rock

sandpipers including 1) did rock and purple sandpipers differentiate as a result of recent glacial cycles, 2) were rock sandpipers isolated in a single glacial refugium or multiple refugia during the LGM with recent population expansion into the current breeding range, and 3) is there a subspecific correspondence between refugial source and expansion areas? I examined these questions by using mismatch distributions, tree based phylogenetic inference, and coalescent techniques to evaluate phylogenetic relationships, population demography, population divergence times, and levels of gene flow.

MATERIALS AND METHODS

DNA sequencing

Whole genomic DNA from the tissues of forty rock sandpipers collected during the breeding season from nine locations (Fig. 2.2, Table 2.1) and four purple sandpipers from two locations were extracted following Glenn (1997). Most of the mitochondrial cytochrome b gene (1,033 bp) and the entire NADH dehydrogenase subunit 2 gene (ND2; 1,041bp) were amplified and cycle-sequenced using four primer pairs per individual. Primers used for cytochrome b included: L14851 (Kornegay et al. 1993), H16064 (Harshman 1996), L15350 (Klicka and Zink 1997), and H15424 (Hackett 1996). Primers used for ND2 were L5216, H6313, H5758, and H5766 (Sorenson et al. 1999). Amplified products were sequenced in both directions using an ABI 373A or 3100 automated sequencer (Applied Biosystems Inc., Foster City, CA). All sequences were deposited in Genbank (Table 2.1).

Neutrality tests, mismatch distributions, and phylogenetic analyses

Tests for neutrality were performed using Tajima's test of selective neutrality (Tajima 1989) in Arlequin 1.1 (Schneider et al. 1997). Arlequin was also used to determine mismatch distributions. All rock sandpiper sequences were grouped together for both analyses to determine whether they had expanded out of a single refugium after the LGM.

A partition homogeneity test was used to verify that the two molecular data sets (cytochrome b and ND2) could be combined for phylogenetic analyses (Farris et al. 1995a, b). The two data sets were not significantly different ($\underline{P} = 0.24$), so the data sets were combined in all analyses. A maximum likelihood analysis was used to construct phylogenetic trees (PAUP 4.0b10; Swofford 2001). The most appropriate model and parameter estimates for likelihood runs were determined using Modeltest 3.06 (Posada and Crandall 1998). The most appropriate model was the general time reversible model with among-site variation approximated using the proportion of invariable sites (GTR+I). Bootstrap support was evaluated by resampling the data matrix 100 times (Felsenstein 1985).

Bayesian inference was also used to develop phylogenetic trees under the same maximum likelihood model as above using MrBayes 2.01 (Huelsenbeck and Ronquist 2001). This technique provides the most probable tree and the posterior probabilities for each clade under a Bayesian framework using a Markov chain Monte Carlo algorithm (Larget and Simon 1999). These posterior probabilities can be used as an alternative statistic to bootstrapping for determining the accuracy of the estimated phylogeny. Also,

Bayesian methods might provide a more reliable estimate of the true phylogeny because, unlike bootstrapping, the posterior probabilities generated are the true probabilities of each clade under the evolutionary models used (Rannala and Yang 1996).

Multiple runs with a random starting tree were used to ensure that the Markov chain converged at the most optimal likelihood values. Trees sampled before the Markov chain reached a plateau were discarded, and the remaining trees were used to approximate the posterior probability of the phylogeny (Huelsenbeck and Ronquist 2001). Trees were then imported into PAUP 4.0b10 (Swofford 2001) and a majority rule consensus tree was created. The posterior probabilities of clades were determined the percentage of occurrence of each clade among all sampled trees (Huelsenbeck and Ronquist 2001).

Dunlin (*Calidris alpina*; Genbank AY156135, 156159) was used as an outgroup in both phylogenetic analyses, because it is thought to be a close relative of rock and purple sandpipers (Borowik and McLennan 1999). Also, curlew sandpiper (*Calidris ferruginea*; Genbank AY156136, 156160) was used as a more distant outgroup.

Coalescent estimates

A coalescent approach was chosen because this technique has been shown to provide accurate results for population parameters when smaller sample sizes are used (Harding 1996, Miura and Edwards 2001). Non-equilibrium based estimates of θ ($2\mu N_e$), divergence times ($T=t/2N_e$), and gene flow ($M=2N_em$) for population pairs were determined using the program Mdiv (Nielsen and Wakeley 2001), where N_e is the effective population size, t is the time to population divergence in generations, m is the

migration rate in number of individuals per generation, and μ is mutation rate per nucleotide per generation. Divergence times were estimated by assuming a mutation rate of $4.14X10^{-5}$ mutations per nucleotide per generation based on molecular clock rates for both total mitochondrial and cytochrome b in birds (~ 2% per MY; Shields and Wilson 1987, Fleischer et al. 1998). A generation time of one year was used as a conservative estimate, given that rock sandpipers molt into full adult breeding plumage in their first spring (Hayman et al. 1986) and evidence strongly suggests that they breed in their first year. Non-equilibrium estimators were used because of the probability that rock sandpipers experienced recent population expansions, bottlenecks, or incomplete lineage sorting and thus would not be in equilibrium between drift and migration. In addition, Griswold and Baker (2002) found that in expanding chaffinch (*Fringilla coelebs*) populations, non-equilibrium based estimators more accurately approximated migration rates than equilibrium-based methods. The Hasegawa, Kishino, and Yano (HKY) model (Hasegawa et al. 1985) of molecular evolution, which takes into account multiple nucleotide substitutions at the same position, nucleotide frequency differences, and any transition/transversion bias, was used to generate Bayesian posterior distributions of T, M, and θ. Parameter estimates were derived from the modes of each distribution, and Bayesian credibility intervals were determined by taking the shortest intervals containing 95% of the posterior distributions. Mdiv also provides an estimate of time to most recent common ancestor (TMRCA), which is not necessarily the same as T ($t/2N_e$; Edwards and Beerli 2000). These two parameters might differ due to gene flow between recently

isolated populations or due to renewed gene flow after populations have been isolated for extended periods.

RESULTS

Neutrality tests and mismatch distributions

The neutrality test was negative (D = -1.1656) and not significantly different from zero (P = 0.126), which supports the assumption of neutrality and is consistent with a species or group of populations that have expanded or experienced a recent bottleneck (Tajima 1989, Rand 1996). However, it is unlikely that this negative value is due to a recent expansion from a single refugium, because the mismatch distribution (not shown) was bimodal suggesting that populations are subdivided. Thus, it is unlikely that rock sandpipers have recently expanded from a single refugium.

Phylogenetic analyses

The 2,074 bp of mtDNA sequence were combined for phylogenetic analysis to produce a single maximum likelihood tree (Fig. 2.3; see Methods). Rock and purple sandpipers are clearly sister species, but divergence estimates predate the Wisconsin glaciation (approximately 3% sequence divergence or 1.5 Mya). Also, each species is clearly monophyletic; however, purple sandpipers from only two locations were examined (Table 2. 1). Bootstrap support for these relationships is high, but within the rock sandpiper clade support was generally poor, probably because few base pair (2 – 4 bp) differences supported individual clades (Fig. 2.3).

Four separate Bayesian analyses with random starting parameters were examined and all analyses converged at similar log likelihood scores (-4,363.86 to -4,378.36).

These likelihood values were similar to those produced during the maximum likelihood analysis (-4,624.547). The initial 218,200 generations of a total of 281,000 generations were discarded, which resulted in a posterior distribution consisting of 62,800 samples. A majority rule consensus tree based on these samples resulted in 6,286 trees. This consensus tree had an identical topology as the maximum likelihood tree but provided better resolution of within the rock sandpiper clade (Fig. 2.3). This might indicate that Bayesian probability methods are more useful than traditional bootstrap analyses in resolving recently diverged populations possibly due to the conservative nature of bootstrap analyses (Murphy et al. 2001, Zhaxybayeva and Gogarten 2002). The tree (Fig. 2.3) suggests that the Commander Island population is sister to all other populations to the east, which indicates that rock sandpipers diverged from a common ancestor with purple sandpipers and likely colonized the north Pacific via Asia.

There is substantial structure within the rock sandpiper clade, but branch lengths are short, indicating relatively recent population divergences. Several clades are supported by >94% posterior probabilities and >50% bootstrap values, including a clade that contains all individuals from St. Matthew Island, a clade that has all rock sandpipers sampled from Nome, and the monophyletic Commander Island clade (Fig. 2.3). However, there is also evidence for incomplete lineage sorting or recent gene flow within many of these clades. The Nunivak Island sample has individuals that are placed throughout the tree, with haplotypes shared with birds from St. Matthew Island, Pribilof Islands, Nome, Izembek Lagoon, and Cape Peirce. An unusual instance of shared haplotypes is that of a rock sandpiper from Cape Peirce that has the same haplotype as

birds from Attu Island. There is also a strong relationship between Adak Island and Izembek Lagoon, with 90% of haplotypes shared between locations (Fig. 2.3).

There is some genetic basis found for the currently described morphologically-based subspecies. The subspecies *ptilocnemis* and *quarta* appear to be monophyletic, but both *tschuktschorum* and *couesi* are paraphyletic and difficult to differentiate using mtDNA sequences alone (Fig. 2.3). This might represent a recent instance of gene flow between these subspecies. However, incomplete lineage sorting cannot be eliminated as a possible contributor to these patterns.

Coalescent estimates

Values for θ and for the respective 95% credibility intervals range from 0.1 (St. Matthew and Pribilof Islands) to 17.0 (Commander Islands and Nunivak Island) for rock sandpiper populations (Table 2. 2). These credibility intervals are very broad, so differences in effective population sizes among populations are difficult to assess. However, the largest values for θ are found for pairwise comparisons between rock sandpipers and purple sandpipers. These values range from 2.7 (Attu Island and purple sandpiper) to 19.8 (Nunivak Island and purple sandpiper). These higher values may reflect the differences in breeding range and effective population size between these two species.

Divergence times and TMRCA were standardized for differences in effective population size by multiplying these pairwise estimates by θ (Griswold and Baker 2002). All TMRCA estimates are almost twice as large as divergence time estimates (Table 2.3);

this is likely due to the maintenance of ancestral polymorphisms or historically high levels of gene flow between now-isolated populations (Edwards and Beerli 2000).

All TMRCA estimates predate the LGM and range from 36,460 ybp (St. Matthew and Pribilof Islands) to 192,574 ybp (Nunivak and Commander Islands). Divergence estimates (T; Table 2.3) are probably a more conservative indicator of population divergence (Nielsen and Wakeley 2001) and suggest that some population pairs diverged at the end of the LGM. These recent estimates include the Adak Island - Izembek Lagoon pair (13,771 ybp) and the Attu Island - Cape Peirce pair (14,545 ybp). Most of the population pairs have divergence times that occurred between 30,000 – 60,000 ybp (Table 2.3). However, two locations consistently have the oldest divergence estimates: Commander Islands (67,702 – 241,731 ybp) and St. Matthew Island (27,936 – 241,731 ybp; Table 2.3). In addition, these two populations are the most divergent from one another (241,731 ybp; Table 2.3). However, all pairwise estimates with the Commander Island population must be viewed with caution. Because of the small sample size from this location ($\underline{n} = 2$) these estimates might be skewed upward. Pairwise estimates between purple and rock sandpipers have divergence dates that predate the Wisconsin glaciation and correspond fairly well with estimates based on percent sequence divergence (approximately 1.5 MYa; Table 2.3).

Gene flow

Estimates of gene flow show very little movement between most population pairs (Table 2.2). Only two pairs have N_em values larger than 1.0 (Izembek Lagoon - Adak Island and Nunivak Island - Nome), which suggests that levels of gene flow between

these populations are sufficient to overcome drift (Hartl and Clark 1997). Once again, credibility intervals are large, possibly due to small sample sizes; however, these gene flow estimates match well with the shared haplotypes shown in the phylogenetic analysis (Fig. 2.3). Large credibility intervals are not necessarily solely a consequence of small sample size, however, as a recent study found equally large credibility intervals when larger sample sizes were used ($\underline{n}$ = 7 - 13 per location; Griswold and Baker 2002). Many of the pairwise estimates have very low values (0.01), suggesting a lack of gene flow between these locations. As expected, this low value was also found for all purple sandpiper and rock sandpiper pairs. These populations have likely not shared individuals for approximately 1.5 MY, but these pairs also have large credibility intervals (0.01 to 5.80 migrants per generation). This suggests that these intervals are skewed upward, possibly due to the maintenance of ancestral polymorphisms, incomplete lineage sorting, or relatively small samples sizes.

DISCUSSION

Rock and purple sandpiper phylogeography

Rock and purple sandpipers are sister taxa that diverged well before the beginning of the Wisconsin glaciation. Based on both percent sequence divergence and coalescence estimates (approximately 1.5 MYa) these species diverged around the beginning of the Pleistocene (1.8 MYa), which corresponds with the onset of many major glaciations (Williams et al. 1998). It is difficult to attribute this divergence to any particular event, but by examining the current distributions of these species a pattern emerges. Both species are coastally distributed, but rock sandpipers are only found in the north Pacific,

and purple sandpipers occur in the north Atlantic. Large expanses of land (North American and Asia) appear to be the primary barrier between these species. Although land might seem to be an unusual barrier to gene flow for birds, both rock and purple sandpipers have limited migratory movements, and their entire lives are spent on or near saltwater beaches (Murie 1959, del Hoyo 1996). The most northern breeding populations of both species are seasonal migrants, and these migratory populations correspond with areas where pack ice reaches the shore in winter (locations in the Bering Sea, the Bering Strait, Siberia, and northern Europe). Thus, both ice and land appear to constrain the present distributions of both species.

Population expansions and contractions

There appear to have been at least three expansions and two intervening range contractions of rock sandpipers during the late Pleistocene (Fig. 2.4). Coalescence estimates (Table 2.3) and the phylogenetic tree (Fig. 2. 3) indicate that the first expansion occurred with colonization of the Pacific from an Asian source population into North America well before the Wisconsin glaciation (ca. 117,000 – 241,000 ybp; Fig. 2.4A). It is likely that this Pacific population expanded broadly, given that the oldest pairwise estimates of divergence (181,806 - 241,731 ybp; Table 2.3) are between two geographically distant populations (Commander Islands and St. Matthew Island). However, coalescent dates (ca. 30,000 – 117,000 ybp) suggest that with the advent of the Wisconsin glaciation, populations probably contracted into two primary areas – a western Pacific refugium that corresponds to the Commander Islands and a refugium probably on the eastern Bering land bridge that corresponds to the current location of St. Matthew

Island (because these two populations have the oldest pairwise divergence estimates with most other rock sandpiper populations (Table 2.3, Fig. 2.4B). However, St. Matthew and the Pribilof Islands show a recent divergence (28,000 ybp), and thus these two populations were likely isolated in the same refugia during this initial range contraction.

A second expansion probably occurred in one or both of the Wisconsin inter-stadials (approximately 25,000 ybp and 75,000 ybp; Williams et al. 1998). Populations from the western Pacific refugium likely expanded into the Aleutian Islands and Alaska mainland, while populations from the Bering land bridge refugium most likely expanded very little within the Bering Sea region (Fig. 2.4B). The phylogenetic tree shows a close association between the Commander Island and Attu Island populations and between St. Matthew Island and the Pribilof Islands (Fig. 2.3). In addition, pairwise estimates of divergence times between Commander Island and Attu Island (ca. 88,000 ybp) are closer than pairwise estimates between this population and St. Matthew Island (142,000 ybp). Likewise, the coalescence estimates between St. Matthew Island and the Pribilof Islands (28,000 ybp) are much more recent than those between the Pribilof Islands and the Commander Islands (ca. 216,000 ybp).

After this expansion (Fig. 2.4B), a less severe range contraction seems to have occurred, with possibly three populations persisting through the LGM in the Bering land bridge region (ca. 30,000 ybp – present), corresponding to the current locations of St. Matthew Island, the Pribilof Islands, and Nome; a population persisting near the Commander Islands; and two populations in the Aleutian Islands (Fig. 2.4C). The final range expansion occurred within the last 12,000 yrs as the glacial ice receded at the end

of the Pleistocene. However, migration and divergence estimates between locations suggest that some populations have not expanded (Commander, St. Matthew, and the Pribilof islands; Tables 2.2, 2.3), while others probably continued to exchange individuals (Adak Island - Izembek Lagoon and Nome - Nunivak Island; Table 2.2).

Refugia

If a single refugium had harbored rock sandpipers through the LGM and subsequent post-glacial colonization had occurred, then the mismatch distributions would have been unimodal (Rogers 1995). However, the distributions were bimodal. In addition, most pairwise coalescence estimates of divergence time predate the LGM, and some even predate the beginning of the Wisconsin glaciation (Table 2.3). These results suggest that rock sandpipers were isolated in several refugia and that these populations experienced different histories of persistence and expansion.

Two areas served as refugia for rock sandpiper populations that persisted throughout the Wisconsin glaciations (117,000 – 10,000 ybp). These refugia correspond to the Commander Islands (the western Pacific refugium) and St. Matthew Island and Pribilof Islands (the Bering land bridge refugia). These populations have the oldest lineages based on coalescence divergence estimates (Table 2.3, Fig. 2.4B), and likely served as sources for all subsequent rock sandpiper populations.

During the LGM (30,000 – 10,000 ybp), three other areas appear to have served as refugia including Nome on the Alaska mainland and Attu Island and Adak Island in the Aleutian Islands (Fig. 2.2, 2.4C). Genetic support for the Nome refugium includes the grouping of the three individuals from Nome in a single well supported clade (Fig.

2.3) and the pairwise divergence estimates that all predate the LGM (Table 2.3). However, an individual from Nunivak Island is also found in the Nome clade which suggests that the Nunivak Island population might have colonized Nome. This scenario seems unlikely because individuals from Nunivak Island are scattered throughout the tree (Fig. 2.3) and the gene flow estimates between Nome and Nunivak Island are >1 individual per generation which indicates that Nunivak was probably colonized by several refugial populations (Fig. 2.4C).

The Aleutian locations also have refugial signatures (Attu and Adak islands; Table 2.3, Fig. 2.4C). These two locations have likely not had gene flow for 56,000 yrs (Table 2.3). However, both locations share haplotypes with different populations on the mainland. Cape Peirce and Attu Island are grouped together in the phylogenetic tree and share one haplotype (Fig. 2.3). However, estimates of gene flow are less than one per generation (Table 2.2), which might indicate that there has been incomplete lineage sorting between these locations. Adak Island shares many haplotypes with Izembek Lagoon and has gene flow estimates of 3 to 4 individuals per generation (Table 2.2). Contemporary gene flow is suggested. Additional support for these two locations as refugia are other studies that have shown that one or both of these locations were refugia for other bird species (Holder et al. 1999, Congdon et al. 2000). Finally, and perhaps not surprisingly, each of the reconstructed expansions of this species within Beringia (Fig. 2.4) reflect a common west-to-east pattern of colonization exhibited among many Asian species ((Pielou 1991, D. Gibson, personal communication).

Rock sandpiper taxonomy

Climate change and the isolation of populations in refugia appear to have caused the pattern of subspecific differentiation evident today. There is strong evidence that the subspecies *quarta* and *ptilocnemis* represent distinctly different evolutionary units that have been isolated for much of the Wisconsin period (Fig. 2.2). These two morphologically-based entities were isolated in the two primary refugia (the western Pacific (*quarta*) and the Bering land bridge (*ptilocnemis*)) that permitted the differentiation of these subspecies. However, *couesi* and *tschuktschorum*, although morphologically separable from the other two subspecies, are impossible to differentiate based on the genetic data presented here. Although isolation in multiple refugia probably caused the morphological variation found in these two subspecies, contemporary gene flow is probably relatively high between these units. An examination of population structure using more rapidly evolving molecular markers might indicate whether the populations on Attu Island and Nome, for example (the former being sedentary and the latter migratory), are currently isolated from other populations.

ACKNOWLEDGMENTS

This project was supported by the University of Alaska Museum, the National Geographic Society, the U.S. Department of Agriculture, the National Science Foundation, and an anonymous donor. Field and technical support were provided by the U.S. Fish and Wildlife Service and the U.S. Coast Guard. I thank the personnel at the Izembek, Togiak, Kodiak, and Alaska Maritime National Wildlife Refuges, Bureau of

Land Management, and the Alaska Department of Fish and Game - Nome for allowing the use of vehicles and facilities. I thank K. Winker, D. D. Gibson, R. W. Dickerman, G. M. Spellman, T. M. Braile, D. A. Rocque, A. B. Johnson, T. M. Boucher, A. Petersen, and S. V. Zagrebelny for technical assistance and field sampling. A purple sandpiper tissue from Louisiana was provided by J. V. Remsen of the Louisiana State Museum of Vertebrate Zoology. For helpful comments and discussion, I thank A. Denton, T. Hahn, K. Schwaegerle, and K. Winker.

REFERENCES

American Ornithologists' Union (AOU). 1910. Check-list of North American Birds, 3rd ed. American Ornithologists' Union, New York.

American Ornithologists' Union (AOU). 1931. Check-list of North American Birds, 4th ed. American Ornithologists' Union, Lancaster, Pennsylvania.

American Ornithologists' Union (AOU). 1957. Check-list of North American Birds, 5th ed. American Ornithologists' Union, Baltimore, Maryland.

Borowik, O. A., and D. A. McLennan. 1999. Phylogenetic patterns of parental care in Calidridine sandpipers. Auk 116:1107-1117.

Congdon, B. C., J. F. Piatt, K. Martin, and V. L. Friesen. 2000. Mechanisms of population differentiation in marbled murrelets: Historical versus contemporary processes. Evolution 54:974-986.

del Hoyo., J., A. Elliott, and J. Sargatal. 1996. Handbook of the birds of the world, Vol 3. Hoatzins to Auks. Lynx Editions, Barcelona, Spain.

Edwards, S. V., and P. Beerli. 2000. Gene divergence, population divergence, and the variance in coalescence time in phylogeographic studies. Evolution 54:1839-1854.

Farris, J. S., M. Kallersjo, A. G. Kluge, and C. Bult. 1995a. Testing significance of incongruence. Cladistics 10:315-319.

Farris, J. S., M. Kallersjo, A. G. Kluge, and C. Bult. 1995b. Constructing a significance test for incongruence. Systematic Biology 44:570-572.

Fay, F. H., and T. J. Cade. 1959. An ecological analysis of the avifauna of St. Lawrence Island, Alaska. University of California Publications in Zoology 63:73-150.

Felsenstein, J. 1985. Confidence limits on phylogenies: An approach using the bootstrap. Evolution 29:783-791.

Fleischer, R. C., C. E. McIntosh, and C. L. Tarr. 1998. Evolution on a volcanic conveyor belt: Using phylogeographic reconstructions and K-Ar-based ages of the Hawaiian Islands to estimate molecular evolutionary rates. Molecular Ecology 7:533-545.

Gibson, D. D., and B. Kessel. 1992. Seventy-four new avian taxa documented in Alaska 1976-1991. Condor 94:454-467.

Gibson, D. D., and B. Kessel. 1997. Inventory of the species and subspecies of Alaska birds. Western Birds 28:45-95.

Glenn, T. 1997. Genetic bottlenecks in long-lived vertebrates: Mitochondrial and microsatellite DNA variation in American alligators and whooping cranes. Ph.D. dissertation, University of Maryland, College Park.

Griswold, C. K., and A. J. Baker. 2002. Time to the most recent common ancestor and divergence times of populations of common chaffinches (*Fringilla coelebs*) in Europe and North Africa: Insights into Pleistocene refugia and current levels of migration. Evolution 56:143-153.

Hamilton, T. D., K. M. Reed, and R. M. Thorsen. 1986. Glaciation in Alaska. Alaska Geological Society, Anchorage, Alaska.

Hackett, S. J. 1996. Molecular phylogenetics and biogeography of tanagers in the genus *Ramphocelus* (Aves). Molecular Phylogenetics and Evolution 5:368-382.

Harding, R. M. 1996. New phylogenies: An introductory look at the coalescent. Pp. 15-22 *in* P. H. Harvey et al., eds. New uses for new phylogenies. Oxford University Press, UK.

Harshman, J. 1996. Phylogeny, evolutionary rates, and ducks. Ph. D. dissertation, University of Chicago, Chicago, Illinois.

Hartl, D. L., and A. G. Clark. 1997. Principles of population genetics, 3rd ed. Sinauer, Sunderland, Massachusetts.

Hasegawa, M. H., H. Kishino, and T. Yano. 1985. Dating of the human-ape splitting by a molecular clock of mitochondrial DNA. Journal of Molecular Evolution 21:160-174.

Hayman, P., J. Marchant, and T. Prater. 1986. Shorebirds: An identification guide to the waders of the world. Houghton Mifflin Company, Boston, Massachusetts.

Hewitt, G. 2000. The genetic legacy of the Quaternary ice ages. Nature 405:907-913.

Holder, K., R. Montgomerie, and V. L. Friesen. 1999. A test of the glacial refugium hypothesis using patterns of mitochondrial and nuclear DNA sequence variation in rock ptarmigan (*Lagopus mutus*). Evolution 53:1936-1950.

Hopkins, D. M. 1967. The Cenozoic history of Beringia – A synthesis. Pp. 451-484 *in* D. M. Hopkins, ed. The Bering land bridge. Stanford University Press, Stanford, California.

Hopkins, D. M., J. V. Matthews, Jr., C. E. Schweger, and S. B. Young. 1982. Paleoecology of Beringia. Academic Press, New York.

Huelsenbeck, J. P., and F. Ronquist. 2001. MR-BAYES: Bayesian inference of phylogeny. Bioinformatics 17:754-755.

Klicka, J., and R. M. Zink. 1997. The importance of recent ice ages in speciation: A failed paradigm. Science 277:1666-1669.

Kornegay, J. R., T. D. Kocher, L. A. Williams, and A. C. Wilson. 1993. Pathways of lysozyme evolution inferred from the sequences of cytochrome b in birds. Journal of Molecular Evolution 37:367-379.

Larget, B., and D. L. Simon. 1999. Markov chain Monte Carlo algorithms for the Bayesian analysis of phylogenetic trees. Molecular Biology and Evolution 16:750-759.

Muira, G. I., and S. V. Edwards. 2001. Cryptic differentiation and geographic variation in genetic diversity of Hall's babbler *Pomastostomus halli*. Journal of Avian Biology 32:102-110.

Murie, O. J. 1959. Fauna of the Aleutian Islands and Alaska Peninsula. North American Fauna No. 61, U. S. Fish and Wildlife Service, Washington D. C.

Murphy, W.J., E. Eizirik, S. J. O'Brien, O. Madsen, M. Scally, C. J. Douady, E. Teeling, O. A. Ryder, M. J. Stanhope, W. W. de Jong, and M. S. Springer. Resolution of the early placental mammal radiation using Bayesian phylogenetics. Science 294:2348-2351.

Nielsen, R., and J. Wakeley. 2001. Distinguishing migration from isolation: A Markov chain Monte Carlo approach. Genetics 158:885-896.

Peters, J. L. 1934. Check-list of the birds of the world. Harvard University Press, Cambridge, Massachusetts.

Pielou, E. C. 1991. After the ice age. University of Chicago Press, Illinois.

Posada, D., and K. A. Crandall. 1998. Modeltest: Testing the model of DNA substitution. Bioinformatics 14:817-818.

Rand, D. M. 1996. Neutrality tests of molecular markers and the connection between DNA polymorphism, demography, and conservation biology. Conservation Biology 10:665-671.

Rannala, B., and Z. Yang. 1996. Probability distribution of molecular evolutionary trees: A new method of phylogenetic inference. Journal of Molecular Evolution 43:304-311.

Rogers, A. R. 1995. Genetic evidence for a Pleistocene population explosion. Evolution 49:608-615.

Schneider, S., J. Kueffer, D. Roessli, and L. Excoffier. 1997. Arlequin ver. 1.1: A software for population genetic data analysis. Genetics and Biometry Laboratory, University of Geneva, Switzerland.

Shields, G. F., and A. C. Wilson. 1987. Calibration of mitochondrial DNA evolution in geese. Journal of Molecular Evolution 24:212-217.

Sorenson, M.D., J.C. Ast, D.E. Dimcheff, T. Yuri & D.P. Mindell. 1999. Primers for a PCR-based approach to mitochondrial genome sequencing in birds and other vertebrates. Molecular Phylogenetics and Evolution 12: 105-114.

Stewart, J. R., and A. M. Lister. 2001. Cryptic northern refugia and the origins of the modern biota. Trends in Ecology and Evolution 16:608-613.

Swofford, D. L. 2001. Phylogenetic analysis using parsimony (and other methods), ver. 4.0b10. Sinauer Associates, Sunderland, Massachusetts.

Tajima, F. 1989. Statistical method for testing the neutral mutation hypothesis of DNA polymorphism. Genetics 123:585-595.

Williams, M., D. Dunkerley, P. De Deckker, P. Kershaw, and J. Chappell. 1998. Quaternary Environments, 2nd ed. Arnold, London, U. K.

Zhaxybayeva, O., and J. P. Gogarten. Bootstrap, Bayesian probability and maximum likelihood mapping: exploring new tools for comparative genome analyses. BMC Genomics 3: 4

Table 2.1. Species, subspecies, sampling locations, and Genbank accessions for rock and purple sandpipers used in this study. Museum voucher numbers are provided on Genbank.

Species	Location	n	Genbank accession	
			cytochrome b	ND2
Calidris ptilocnemis quarta	Commander Is., Russia	2	AY156153 - 154	AY156129 - 130
C. p. couesi	Attu Is., Aleutian Is., Alaska	5	AY156101 - 105	AY156223 - 227
C. p. couesi	Adak Is., Aleutian Is., Alaska	5	AY156233 - 237	AY156091 - 095
C. p. tschuktchorum	Izembek Lagoon, Alaska Pen., Alaska	5	AY156247 - 251	AY156109 - 113
C. p. tschuktchorum	Togiak NWR, Cape Peirce, Alaska	5	AY156254 - 258	AY156124 - 128
C. p. tschuktchorum	Nunivak Is., Alaska	5	AY156137 – 141	AY156096 - 100
C. p. tschuktchorum	Nome, Seward Pen., Alaska	3	AY156142, 252 – 253	AY156106 - 108
C. p. ptilocnemis	St. Matthew Is., Alaska	5	AY156143 – 147	AY156114 - 118
C. p. ptilocnemis	Pribilof Is., Alaska	5	AY156148 – 152	AY156119 - 123
C. maritima	Reykjavik, Iceland	3	AY156155 - 157	AY15131 - 133
C. maritima	Cameron Parrish, Louisiana	1	AY156158	AY156134

Table 2.2. Mdiv estimates of 0 ($2 N_e\mu$) above diagonal and N_em below diagonal of population pairs for rock and purple sandpipers. Values in parentheses are 95% credibility intervals. See Fig. 1 for population locations.

	Commander	Attu	Adak	Izembek	C. Peirce	Nunivak	Nome	St. Matt.	Pribilofs	*maritima*
Commander Is.	----	1.54 (0.4–8.3)	3.38 (1.2-12.0)	2.41 (0.5-9.5)	2.57 (0.7-12.2)	5.49 (1.9-17.0)	2.16 (0.6-9.9)	1.73 (0.6-8.5)	1.75 (1.0-12.2)	8.53 (3.2-14.5)
Attu Is.	0.01 (0.01-5)	----	2.41 (0.6-9.1)	1.07 (0.2-5.5)	1.16 (0.2-7.0)	3.29 (0.9-11.3)	1.02 (0.3-5.5)	1.23 (0.3-6.1)	1.08 (0.2-5.3)	4.61 (2.7-12.1)
Adak Is.	0.03 (0.01-5)	0.05 (0.01-5)	----	1.78 (0.5-8.3)	2.96 (0.8-11.1)	5.06 (1.9-15.7)	2.50 (0.7-10.2)	2.18 (0.7-10.4)	1.99 (0.6-9.5)	7.25 (4.0-16.5)
Izembek NWR	0.01 (0.01-5)	0.01 (0.01-5)	3.32 (0.45-5)	----	1.63 (0.4-8.1)	3.41 (1.2-12.5)	1.46 (0.3-6.7)	1.60 (0.4-7.1)	1.07 (0.3-6.3)	5.01 (3.0-13.2)
Cape Peirce	0.01 (0.01-5)	0.16 (0.01-5)	0.09 (0.01-5)	0.03 (0.01-5)	----	3.36 (1.0-12.2)	1.61 (0.3-8.7)	1.58 (0.4-9.4)	1.43 (1.9-15.7)	5.17 (2.9-15.4)
Nunivak Is.	0.08 (0.01-5)	0.35 (0.01-5)	0.52 (0.01-5)	0.30 (0.01-5)	0.63 (0.15-5)	----	2.68 (0.9-11.1)	3.00 (0.7-12.3)	2.33 (0.6-11.2)	7.31 (4.1-19.8)
Nome	0.03 (0.01-5)	0.01 (0.01-5)	0.01 (0.01-5)	0.01 (0.01-5)	0.01 (0.01-5)	1.09 (0.27-5)	----	1.28 (0.3-6.7)	0.99 (0.2-4.4)	5.29 (2.8-12.2)
St. Matthew Is.	0.01 (0.01-5)	0.01 (0.01-5)	0.02 (0.01-5)	0.01 (0.01-5)	0.01 (0.01-5)	0.17 (0.01-5)	0.01 (0.01-5)	----	0.46 (0.1-3.0)	5.15 (3.0-12.1)
Pribilof Is.	0.01 (0.01-5)	0.01 (0.01-5)	0.01 (0.01-5)	0.01 (0.01-5)	0.01 (0.01-5)	0.26 (0.03-5)	0.01 (0.01-5)	0.14 (0.01-5)	----	4.75 (2.8-11.0)
maritima	0.01 (0.01-5)	0.01 (0.01-5)	0.01 (0.01-5)	0.01 (0.01-5)	0.01 (0.01-5)	0.01 (0.01-5)	0.01 (0.01-5)	0.01 (0.01-5.8)	0.01 (0.01-5)	----

Table 2.3. Mdiv estimates (corrected for unequal N_e by multiplying by θ) of T ($t/2Ne$) above diagonal and time to most recent common ancestor (TMRCA) below diagonal of population pairs for rock and purple sandpipers. Values in parentheses are 95% credibility intervals of T. Bottom numbers are years before present assuming a generation time of one year. Mdiv does not provide credibility intervals for TMRCA estimates. See Fig. 1 for population locations.

	Commander	Attu	Adak	Izembek	C. Peirce	Nunivak	Nome	St. Matt.	Pribilofs	*maritima*
Commander Is.	----	3.64 (0.7-7.7) 87,950	3.51 (1.3-16.9) 84,898	4.72 (1.4-24.1) 114,087	2.80 (1.0-12.9) 67,702	4.55 (0.7-27.4) 109,979	8.11 (0.9-21.6) 195,912	10.01 (1.5-17.3) 241,731	8.96 (0.1-8.7) 216,417	122.79 (7.7-128) 2,965,915
Attu Is.	4.25 106,938	----	2.33 (0.9-12.0) 56,352	1.31 (0.52-5.3) 31,758	0.60 (0.12-5.4) 14,545	1.51 (0.6-16.4) 36,550	1.85 (0.5-5.1) 44,761	5.90 (0.6-6.2) 142,519	3.81 (0.5-5.4) 91,968	68.93 (5.6-69.2) 1,665,027
Adak Is.	6.64 160,396	4.08 98,574	----	0.57 (0.04-8.9) 13,771	1.78 (0.5-14.8) 42,941	2.48 (0.7-25.3) 59,870	1.65 (0.6-12.5) 39,842	4.09 (1.1-10.9) 98,674	2.70 (1.0-9.9) 65,214	54.97 (5.7-72.5) 1,327,788
Izembek NWR	6.72 162,401	2.52 60,790	2.86 69,154	----	1.27 (0.5-8.1) 30,689	1.43 (0.4-17.1) 34,599	3.11 (0.6-7.3) 75,005	3.64 (0.7-8.0) 87,917	2.41 (0.5-5.3) 58,095	70.40 (6.0-75.1) 1,700,406
Cape Peirce	5.93 143,174	3.56 85,897	4.73 114,354	3.63 87,796	----	2.72 (0.1-16.8) 65,730	2.06 (0.7-8.1) 49,796	6.46 (0.8-7.9) 156,136	2.02 (0.2-7.1) 48,886	58.38 (5.4-77.4) 1,420,040
Nunivak Is.	7.93 192,574	5.93 143,201	6.06 146,442	5.30 128,048	5.86 141,451	----	6.07 (0.03-13.4) 146,534	3.90 (0.5-15.0) 94,304	3.48 (0.3-11.7) 83,966	66.02 (9.4-109.7) 1,594,786
Nome	6.88 166,300	3.24 78,280	4.11 99,251	3.50 84,447	4.28 103,363	5.35 129,248	----	4.19 (0.4-6.4) 101,131	2.65 (0.4-5.0) 64,114	66.02 (5.6-79.3) 1,594,605
St. Matthew Is.	7.53 181,806	5.01 121,073	5.48 132,387	5.48 132,259	5.36 129,374	6.25 150,946	4.67 112,723	----	1.16 (0.1-2.32) 27,936	73.21 (6.2-77.2) 1,768,286
Pribilof Is.	7.35 177,650	4.25 102,573	4.41 106,541	3.41 82,396	4.57 110,460	4.88 117,793	3.48 84,123	1.51 36,460	----	70.63 (4.8-71.2) 1,705,947
maritima	66.42 1,604,343	51.19 1,236,556	56.45 1,363,544	52.38 1,265,147	51.03 1,232,607	52.57 1,269,902	51.44 1,242,445	59.49 1,436,874	55.14 1,331,804	----

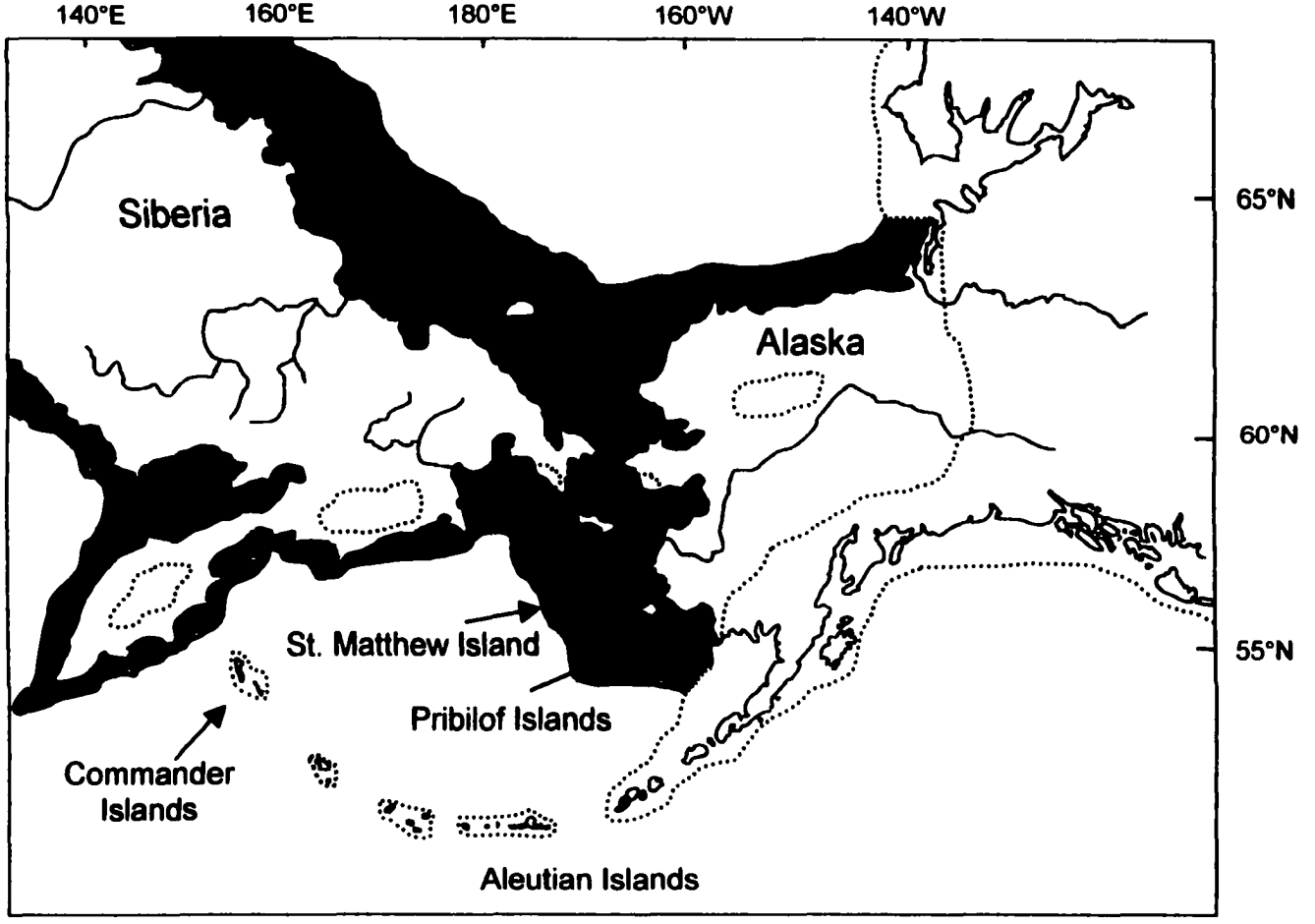

Fig. 2.1. Map of Beringia including the extent of the Bering land bridge (shaded in gray), present sea levels or coastlines, and areas glaciated during the last glacial maximum (areas outlined with dashed lines).

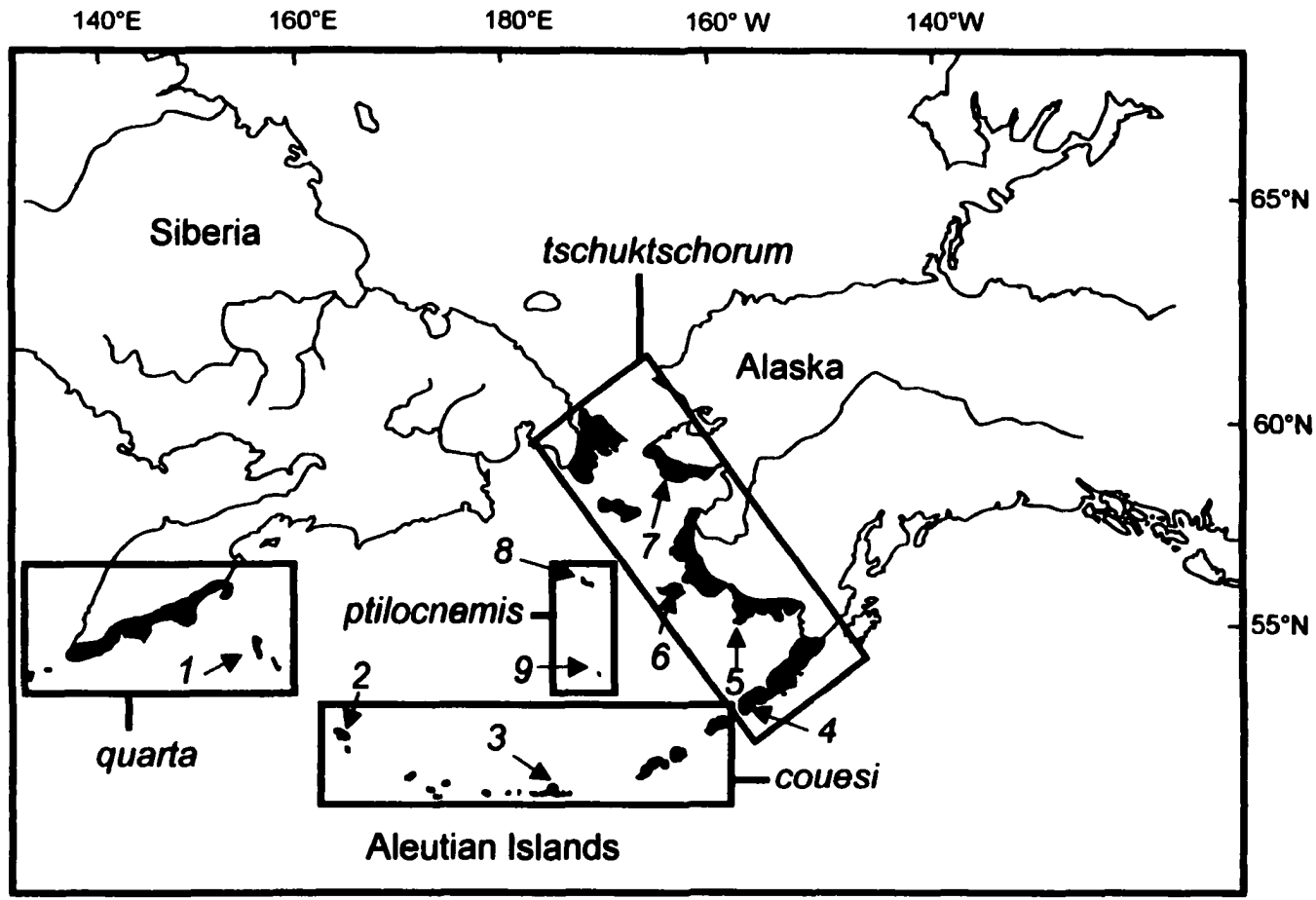

Fig. 2.2. Map of the current subspecies breeding ranges of rock sandpipers and the collection locations of birds used in this study. Numbers correspond to specific localities 1) Commander Islands, 2) Attu Island, 3) Adak Island, 4) Izembek Lagoon, 5) Cape Peirce, 6) Nunivak Island, 7) Nome, 8) St. Matthew Island, and 9) Pribilof Islands. Shaded areas show current rock sandpiper breeding distribution.

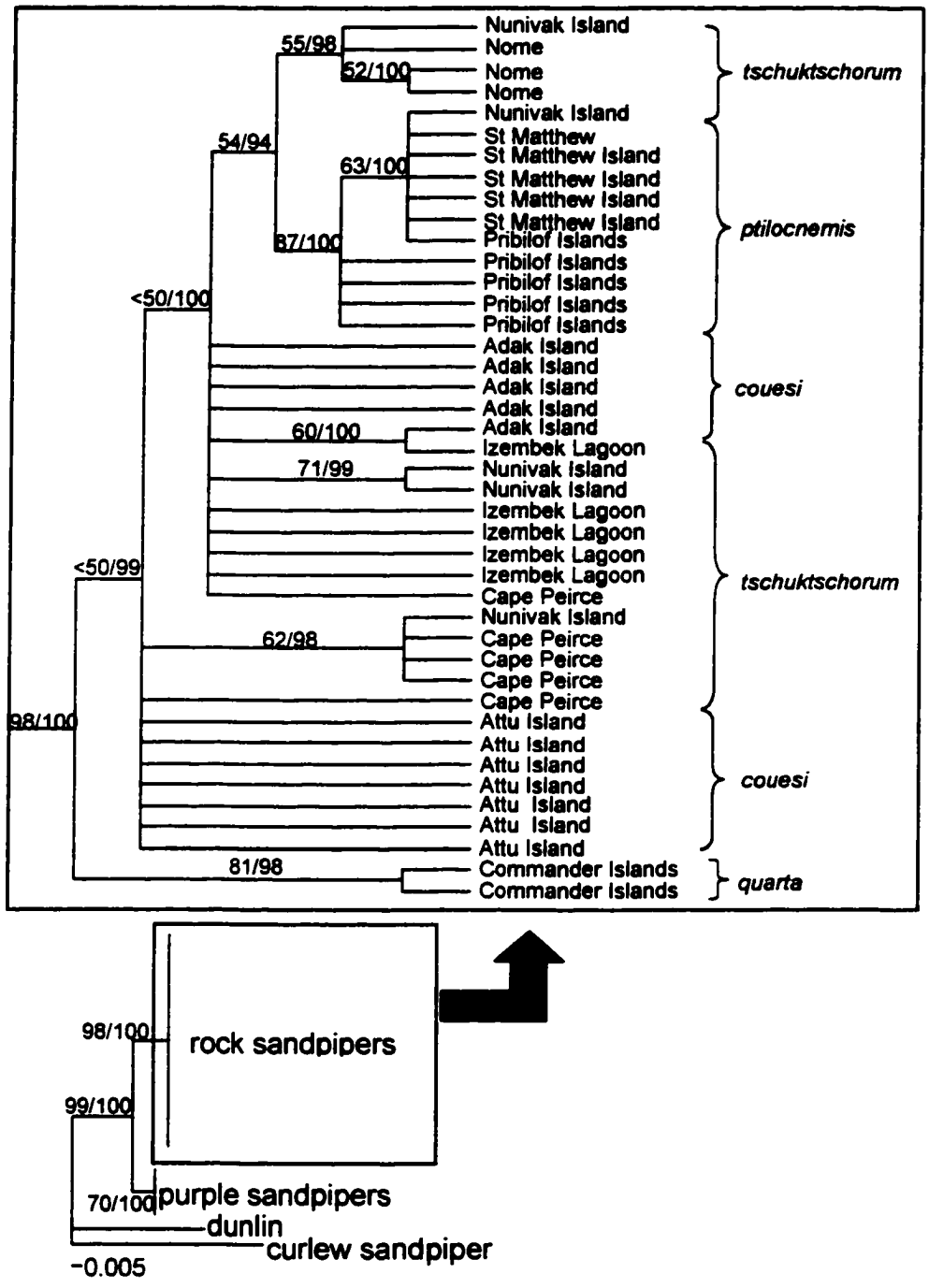

Fig. 2.3. Maximum likelihood and Bayesian trees of the phylogenetic relationships of rock sandpipers based on mitochondrial cytochrome b and ND2 sequences (2,074 bp) and the subspecies associated with each population. Smaller phylogram (below) shows relative branch lengths for each species, while larger tree shows relationships within rock sandpipers based on Bayesian majority rule consensus tree with bootstrap (first number) and posterior probability (second number) values above each branch. Bayesian tree does not show relative branch lengths.

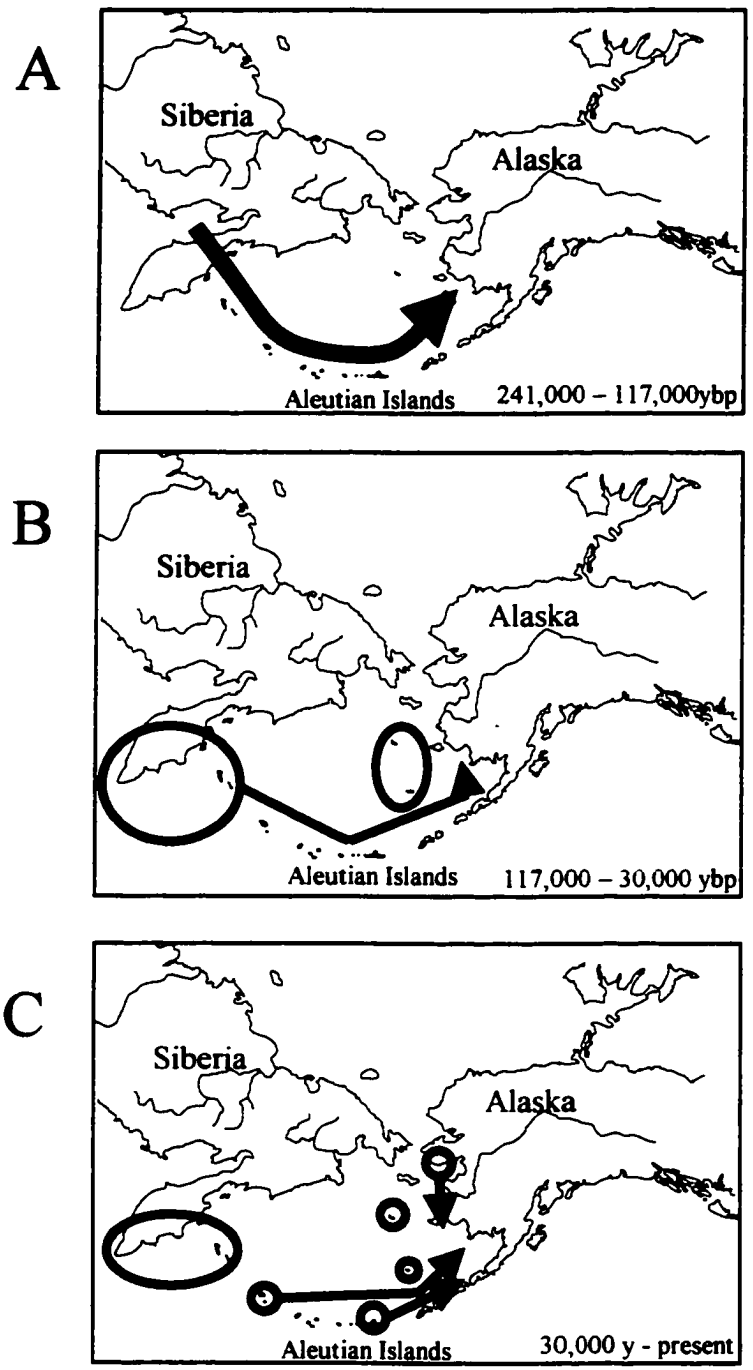

Fig. 2.4. Maps of the expansions, contractions, and suggested refugia of rock sandpiper populations across three time periods. Ovals represent refugial areas, and arrows represent directions of population expansion. A) arrow represents the initial expansion from Asia into North America, B) represents secondary expansions during interstadials (>30,000 ybp), and C) represents recent (<30,000 ybp) population isolation, contraction, and subsequent post-glacial expansion.

VIII. Chapter 3

The genetics of sequentially peripheral isolates and the giant song sparrows of Alaska[3]

ABSTRACT

Island populations at the periphery of species' ranges are likely to exhibit morphological variation and losses of genetic diversity due to a variety of processes, including divergent selection, genetic drift, restricted gene flow, and founder effects. Several factors make Alaska song sparrows useful for evaluating these processes in a natural vertebrate system. Song sparrow populations at the northwestern limits of the species' range are non-migratory, occupy different habitats, and are morphologically divergent from mainland populations. I studied the genetics of eight populations (N = 208) ranging from Attu Island (the species' most western population) to the Queen Charlotte Islands in British Columbia. I used mtDNA sequences and nuclear microsatellite loci to examine the genetic effects of sequential peripheral isolation. I found that northwestern populations of song sparrows appear to have followed a simple stepping-stone model of colonization from east-to-west approximately 30,000 ybp. In addition, molecular evidence suggests that both historic (isolation in multiple glacial refugia, high levels of gene flow of greater than one individual per generation, and small effective population sizes), and current factors (low genetic diversity and limited gene flow) have affected song sparrow populations. The evolution of differences in morphology and behavior of song sparrow populations occurred

[3] Pruett, C. L., and K. Winker. (in preparation). The genetics of sequentially peripheral isolates and the giant song sparrows of Alaska. Evolution.

between the neutral time estimates of mtDNA and microsatellite evolution. This suggests that the examination of molecular markers that evolve at different rates can provide insight into the processes that lead to subspecies differentiation or the initial stages of speciation.

INTRODUCTION

Populations at the edge of a species' distribution are often small, isolated, and have limited gene flow with central populations (Mayr 1963, Futuyma 1998). In addition, these populations often are found in habitats that are thought to be ecologically marginal for the species (Mayr 1963, Lesica and Allendorf 1995), and thus probably experience strong selection. Through the combined processes of low gene flow, genetic drift, and divergent selection, these peripheral populations are likely to exhibit lower genetic diversity, lower effective population sizes, and pronounced morphological differentiation than central populations (Mayr 1982, Cohan and Hoffman 1986, Futuyma 1998).

Populations on islands can be thought of as quintessential peripheral isolates, in that they are affected by the same processes but have more stringent barriers to gene flow and are likely to have experienced founder events (Cox and Moore 1993, Grant 1998, Emerson 2002). Island populations are often centers of rapid evolutionary change, and some common morphological and behavioral patterns are exhibited by many island populations, including reduction in dispersal capabilities and increases in body size or gigantism (Grant 1998). Founder effects and population bottlenecks may contribute to these changes in both island and peripheral populations because of reductions in effective

population size and consequent increases in the effects of genetic drift coupled with different selection pressures (Nei et al. 1975, Futuyma 1998, Grant 1998). An examination of sequentially isolated populations at increasingly greater distances from a species' main distribution would provide insight into how these processes affect naturally occurring populations.

Song sparrows as a model

Northwestern populations of song sparrows (*Melospiza melodia*) are an excellent case for examining the combined genetic effects of peripheral and island isolation on stable, natural vertebrate populations. Song sparrows are endemic to North America, with the most northern and western populations distributed in a narrow strip along the Pacific coast and islands of Alaska (Fig. 3.1). The linear distribution of these Alaska populations extends for many thousands of kilometers, and they can be thought of as beads on a string that are increasingly distant from the species' continental distribution (Fig. 3.1). This distribution allows for direct examination of some very simplified models of gene flow in a natural system, such as the stepping-stone model in which populations only exchange migrants with neighboring populations, or the island model in which populations can exchange migrants with any other population (Futuyma 1998).

The farthest west song sparrow populations are found on the treeless Aleutian Islands and the Alaska Peninsula, and they occur in a different habitat than other song sparrow populations in Alaska, which tend to be found in heavily forested areas (Murie 1959, Aldrich 1984). These treeless western regions might be thought of as marginal for this species (Murie 1959), and thus these populations might be under very strong

selection pressures. Also, these populations are not seasonally migratory, while most other song sparrow populations in Alaska and elsewhere are at least partially migratory (AOU 1957, Paynter 1970, Gibson and Kessel 1997).

Song sparrows also exhibit remarkable geographic variation in morphology, with seven (Gibson and Kessel 1997) to eight (Paynter 1970) subspecies described (Fig. 3.2) in Alaska. In the most peripheral populations, described as the subspecies *maxima* and *sanaka* (Fig. 3.2), body sizes are very large, showing masses of 40 - 55 g (Rising 1996, personal observations). Other populations weigh on average half that amount (Rising 1996). Thus, these Aleutian populations appear to exhibit many of the traits associated with island isolation, including enlarged body sizes and non-migratory behavior (Grant 1998). Past studies with (only limited sampling in Alaska) attempted to explain this morphological variation using molecular markers and found that peripheral populations were similar to many more centrally located populations, and that the genetic variability exhibited was not partitioned geographically (Hare and Shields 1992, Zink and Dittman1993, Fry and Zink 1998). These researchers hypothesized that recent population expansions, historically high levels of gene flow, rapid morphological differentiation due to strong directional selection that outpaced the rate of evolution in mtDNA, or environmental effects on morphology (James 1983) might explain the patterns found.

I sought to examine the genetic effects of varying degrees of isolation on these naturally occurring vertebrate populations and in doing so test hypotheses based on past research with increased sample sizes from locations throughout the distribution of song sparrows in this region. I used both mtDNA sequences and microsatellite loci to evaluate

population distinctiveness, genetic diversity, effective population sizes, and gene flow among northwestern song sparrow populations. I examined several questions to gain insight into the processes that shape sequentially peripheral isolates including: 1) from what directions and when did song sparrows colonize the western parts of their range; and, based on these results, how rapidly did morphological and behavioral differences accumulate; 2) what are the historic and current levels of gene flow among these populations, and do they agree with stepping stone or island models of gene flow; 3) are there losses in genetic diversity in the most peripheral populations, and if so is there evidence for continuing population bottlenecks; and 4) what long-term effective population sizes have enabled the persistence of these peripheral populations to present? In addition to having theoretical relevance to the genetics of natural systems, these questions are important to conservation genetics in an anthropogenically modified world where habitats are increasingly fragmented.

MATERIALS AND METHODS

DNA sequencing and microsatellite data acquisition

I used mtDNA sequences and microsatellite loci to examine song sparrow populations because these markers are known to evolve at different rates (Shields and Wilson 1987, Fleischer et al. 1998, McDonald and Potts 1997), and thus both contemporary and historical effects of isolation can be examined. Whole genomic DNA from the tissues of 208 song sparrows from eight western breeding populations (Fig. 3.2, Table 3.1) and one location in Massachusetts (Table 3.1) were extracted following Glenn (1997). The Queen Charlotte Island population in British Columbia (Fig. 3.2, Table 3.1)

was included because it has been identified as a possible glacial refugial source for Alaska populations (Zink and Dittman 1993). This sampling scheme from the Queen Charlotte Islands to Attu Island is equivalent to the distance between the U. S. states of Florida and California, which encompasses the breadth of the continental portion of this species' breeding range (Fig. 3.1). Most of the mitochondrial cytochrome b gene (1,137 bp) was amplified and cycle-sequenced using four primer pairs per individual for a subset of the extracted tissues (Table 3.1). Primers used included: L14851 (Kornegay et al. 1993), H16064 (Harshman 1996), L15350 (Klicka and Zink 1997), and H15424 (Hackett 1996). Amplified products were sequenced in both directions using an ABI 373A or 3100 automated sequencer (Applied Biosystems Inc., Foster City, CA). All sequences were deposited in Genbank (Table 3.1).

Eight microsatellite loci were amplified for all individuals using fluorescent dye-labeled primers developed for song sparrows (Jeffery et al. 2001) and for two other bird species (*Escu1*; Hanotte et al. 1994, *GF5*; Petren 1998) and were then genotyped using an ABI 373A or 3100 automated sequencer. Because two of the loci are sexed linked, I examined the proportion of males to females for each population and determined that all populations had roughly equivalent ratios of 3 males to 1 female, so these two loci were included in all analyses.

Phylogenetic analyses and MtDNA coalescence estimates

A maximum likelihood analysis of the sequence data was used to construct phylogenetic trees (PAUP* 4.0b10; Swofford 2001). The most appropriate model and parameter estimates for likelihood runs were determined using Modeltest 3.06 (Posada

and Crandall 1998). The most appropriate model was the general time reversible model with among-site variation approximated using the proportion of invariable sites (GTR+I). Bayesian inference was also used to develop phylogenetic trees under the same maximum likelihood model using MrBayes 2.01 (Huelsenbeck and Ronquist 2001). This technique provides the most probable tree and the posterior probabilities for each clade under a Bayesian framework using a Markov chain Monte Carlo algorithm (Larget and Simon 1999). These posterior probabilities can be used as an alternative statistic to bootstrapping for measuring the accuracy of the estimated phylogeny (Rannala and Yang 1996). Four separate runs with a random starting tree were used to ensure that the Markov chain converged at the most optimal likelihood values. All analyses converged at similar log likelihood scores (-2036.88 to -2074.21). These likelihood values were similar to those produced during the maximum likelihood analysis (-2065.71). Trees sampled before the Markov chain reached a plateau were discarded (the first 480,000 generations out of a total of 630,000 generations), and the remaining trees (15,021 trees) were used to approximate the posterior probability of the phylogeny (Huelsenbeck and Ronquist 2001). Trees were then imported into PAUP* 4.0b10 (Swofford 2001) and a majority rules consensus tree was created. The posterior probabilities of clades were determined by examining the percent of occurrence of each clade among all sampled trees (Huelsenbeck and Ronquist 2001). Swamp sparrow (*Melospiza georgiana*) and Lincoln's sparrow (*Melospiza lincolnii*) were used as outgroups in both phylogenetic analyses, because these species are the closest relatives of song sparrows (Zink and Blackwell 1996; Genbank AY156181 - 182).

Using mtDNA sequence data, non-equilibrium based estimates of θ ($2\mu N_e$), divergence times ($T=t/2N_e$), and gene flow (N_em) for population pairs were determined using the program Mdiv (Nielsen and Wakeley 2001), where N_e is the effective population size, t is the time to population divergence in generations, m is the migration rate in number of migrant individuals per generation, and μ is mutation rate per nucleotide per generation. Divergence times were estimated by assuming a mutation rate of 2.31×10^{-5} mutations per nucleotide per generation, based on molecular clock rates for both total mtDNA and cytochrome b in birds (~ 2% per MY; Shields and Wilson 1987, Fleischer et al. 1998). A generation time of one year was used as a conservative estimate, given that song sparrows molt into full adult breeding plumage in their first spring (Rising 1996), and evidence strongly suggests that they breed in their first year (personal observations). Non-equilibrium estimators were used because of the probability that song sparrows experienced recent population expansions, bottlenecks, or incomplete lineage sorting and thus would not be in equilibrium between drift and migration. In addition, Griswold and Baker (2002) found that in expanding chaffinch (*Fringilla coelebs*) populations, non-equilibrium-based estimators more accurately approximate migration rates than equilibrium-based methods. The Hasegawa, Kishino, and Yano (HKY) model (Hasegawa et al. 1985) of molecular evolution, which takes into account multiple nucleotide substitutions at the same position, nucleotide frequency differences, and any transition/transversion bias, was used to generate Bayesian posterior distributions of T, N_em, and θ. Parameter estimates were derived from the modes of each distribution, and

Bayesian credibility intervals were determined by taking the shortest intervals containing 95% of the posterior distributions.

Microsatellite data analyses

Tests for Hardy-Weinberg equilibrium and linkage disequilibrium were performed using GDA (Lewis and Zaykin 2001). Two measures of genetic diversity were examined, average expected heterozygosity and the mean number of alleles per locus for each population using BIOSYS-1 (Swofford and Selander 1981). Expected heterozygosities were examined because this value combines information from allele frequencies and number of alleles. Expected heterozygosities are also commonly used to examine genetic variation, and its behavior following a bottleneck is well understood (Nei et al. 1975, Keller 2001). One-way *t*-tests were used to determine whether there were significant differences between neighboring populations (those populations geographically nearest) for both measures. A genetic distance tree (Nei 1978) was created using GDA (Lewis and Zaykin 2001) using the Massachusetts sample as a root (Table 3.1)

Gene flow (N_em) and θ ($4N_e\mu$) estimates were determined using the program Migrate (Beerli and Felsenstein 1999). This program uses a maximum likelihood coalescent approach to examine all possible migration events and genealogies, and thus should be superior to traditional F_{st} estimates of gene flow (Beerli and Felsenstein 1999). Also, Migrate provides estimates of both immigration and emigration between pairs of populations, and thus directional gene flow can be determined. Estimates of effective

population size (N_e) were determined from θ values by using a standard microsatellite mutation rate of $1x10^{-3}$ (Hancock 1999).

Assignment tests were used to determine whether sampled individuals were unlikely to be genetically from their population of origin (Cornuet et al. 1999). This method is an indirect means of examining recent instances of gene flow and is robust to violations of Hardy-Weinberg equilibrium. These analyses used likelihood-based Bayesian methods with 10,000 simulated individuals per population with the probability of exclusion threshold set to $P < 0.01$ (GeneClass; Cornuet et al. 1999).

Populations were also tested using the program BOTTLENECK (Piry et al. 1999) to determine whether they had undergone a recent bottleneck. This test uses a simulated coalescent approach to determine whether the observed heterozygosity is larger than the expected heterozygosity given the observed allele number under mutation drift equilibrium. The Wilcoxon test was used to examine the statistical significance of deviation from expected heterozygosity (because fewer than 20 loci were examined), and the stepwise mutation model was used as recommended by Cornuet and Luikart (1996).

RESULTS

Phylogenetic analyses

The maximum likelihood tree showed very little population structure with short branch lengths (Fig. 3.3). This suggests that there was recent colonization of this part of the range of song sparrows. The structure exhibited fits the expected colonization direction of this part of the species' range, because an individual from the Alexander Archipelago in southeast Alaska has the most basal haplotype (Fig. 3.3). All individuals

from the subspecies *maxima* grouped together in a moderately supported clade that also included one individual from the neighboring subspecies *sanaka* (Figs. 3.1, 3.2). All other members of *sanaka* were found in a large clade that included almost all other song sparrows, even the two individuals from Massachusetts. A well-supported clade within the large clade had four individuals, two each from the Kodiak Island and Queen Charlotte Island populations. This is an unusual grouping, given that the two locations are geographically distant (Fig. 3.2).

MtDNA coalescence estimates

Pairwise estimates of θ ($2\mu N_e$) based on 1,137bp of the mtDNA cytochrome b gene were similar, which suggests that effective sizes (N_e) of the sampled populations were similar given a uniform mutation rate (Table 3.2). Pairwise comparisons of population divergence (θ) ranged from 0.26 (Adak Is – Alaska Peninsula) to 3.51 (Hyder – Alexander Archipelago; Table 3.2). Western populations (Attu Is., Adak Is., and Alaska Peninsula) consistently had the lowest θ values when compared with each other, while more eastern populations (Copper River Delta, Hyder, Alexander Archipelago, and Queen Charlotte Is.) had higher values (Table 3.2).

MtDNA gene flow estimates (N_em) for several population pairs were greater than 1.0 (Table 3.2), which suggests levels of gene flow high enough to limit genetic divergence between these populations (Hartl and Clark 1997). These higher rates were primarily found between eastern populations (Copper River Delta, Hyder, Alexander Archipelago, and Queen Charlotte Islands). However, Kodiak Island also showed high inferred levels of gene flow with eastern populations, including the highest value

observed between any population pair (N_em = 4.45; Kodiak Is – Queen Charlotte Is.). The two Aleutian populations (Attu and Adak islands) also showed an estimate of gene flow of >1 individual per generation (N_em = 3.91). Only the Alaska Peninsula population had consistently low gene flow estimates (all <0.64) when compared with all other populations (Table 3.2).

Estimates of divergence time ($T=t/2N_e$) ranged from 0.01 (Kodiak and Queen Charlotte Islands) to 17.05 (Hyder and Queen Charlotte Islands; Table 3.3). The highest estimate was much larger than the next largest value (T = 3.03; Attu Is. – Hyder), which might indicate inflation by high estimates of θ for the Hyder – Queen Charlotte Islands population pair. These estimates correspond to divergence dates ranging from 537 – 738,286 years before present (ybp). Most population pairs had estimates preceding the end of the last glacial maximum, which occurred 10,000 – 12,000 ybp (Table 3.3). Only five population pairs had relatively recent divergence dates (<10,000 ybp: Attu - Adak Island, Alaska Peninsula - Kodiak Island, Alaska Peninsula - Copper River Delta, Alaska Peninsula - Queen Charlotte Islands, and Kodiak Island - Queen Charlotte Islands).

Microsatellite analyses

Tests for Hardy-Weinberg equilibrium showed that two loci (Mme1 from Attu Islands and Mme2 from Kodiak Island) were deficient in heterozygotes after adjustments for multiple comparisons. All loci were in linkage equilibrium.

Populations at the periphery of the species' distribution in Alaska had significantly lower mean expected heterozygosities than neighboring populations (Fig. 3.4). Attu Island had both the lowest heterozygosity and allelic diversity values, while

populations in southeast Alaska and northern British Columbia had similar high expected heterozygosities. The Alexander Archipelago population had the highest mean number of alleles per locus, probably due to the fact that this population sample comprised a grouping of several islands within the archipelago (Table 3.1, Fig. 3.4).

The neighbor-joining tree, based on Nei's (1978) genetic distance (Fig. 3.5), showed two major groupings, one with the three populations in southeast Alaska and British Columbia and the other having all of the western mainland and Aleutian Island populations (Fig. 3.5). The western populations showed a sequential increase in genetic distance from east-to-west (Fig. 3.5), with the most distant population from the Massachusetts root being Attu Island. When multiple populations were sampled within one subspecies, these populations grouped together. These included Attu and Adak island populations within the subspecies *maxima* and the Alexander Archipelago and Queen Charlotte Islands within the subspecies *rufina* (Fig. 3.5).

Microsatellite-based estimates of gene flow (N_em) were all below one, indicating very low to no contemporary gene flow among these song sparrow populations (Table 3.4). Because all estimates were low, the direction of any gene flow is difficult to determine; however, the Copper River Delta and Alexander Archipelago populations appear to be receiving more immigrants than other populations (N_em = 0.19 – 0.42). Estimates of θ ($4\mu N_e$) show that all populations have very similar values (Table 3.5). Given a uniform mutation rate, these estimates correspond to similar, low effective population sizes (N_e of 167-208) for every sampled population (Table 3.5).

Assignment tests corresponded loosely with gene flow estimates (Tables 3.4, 3.6). However, some estimates of the numbers of individuals unlikely to have come from the sampled location were much higher than the gene flow estimates acquired using a coalescence approach, including five individuals excluded from the Alexander Archipelago population and six individuals excluded from the Queen Charlotte Islands. These values seem to correspond better to gene flow estimates from the mtDNA data. This might indicate that current gene flow between southeast Alaska and British Columbia populations is higher than would be expected given the very low coalescent-based estimates. However, other nearby, non-sampled populations might be exchanging genes with these populations. This idea is supported by the genotypes that did not assign to any population (Table 3.6). None of the genotypes from Attu Island were excluded from that population, which indicates very low or non-existent contemporary gene flow between this location and any other song sparrow population (Table 3.6). Other populations showed values suggesting higher levels of contemporary gene flow (N_e of 2 – 6 individuals per sampled population; Table 3.6).

Western populations of song sparrows examined did not show evidence of a recent bottleneck. None of the Wilcoxon tests for heterozygote excess or deficiencies showed a bottleneck signature ($P > 0.05$). In addition, all populations had L-shaped distributions of alleles, indicating that they are in mutation drift equilibrium (Piry et al. 1999).

DISCUSSION

A loss of genetic diversity is expected in peripheral populations, and this is readily apparent in western song sparrows. However, these reductions in diversity are not due to recent bottlenecks, but probably are due to historic founder events or protracted isolation in small, isolated populations. By examining mtDNA sequences and microsatellite loci these intertwined processes can be examined separately. Because of their different rates of mutation, these two classes of markers provide different levels of resolution in discerning the historical processes involved in the establishment and persistence of these sequentially peripheral populations, including varying historical degrees of association with their neighboring populations. These temporal differences can provide insight into the rate of morphological and behavioral responses of Aleutian Island song sparrow populations to selection in the extreme habitats in which they are found.

Colonization of peripheral populations

As expected, song sparrows colonized Alaska from the continental distribution going from east to west as evidenced by the sequence-based tree and the microsatellite distance tree (Figs. 3.3, 3.5). Divergence estimates provide tentative dates of colonization of between 29,000 and 72,000 ybp (Table 3.3). These values are based on comparisons of Adak Island - Alaska Peninsula and Adak Island - Hyder populations, because the Attu Island population shows signs of being recently colonized from Adak Island (Table 3.3). Previous studies hypothesized a recent range expansion of song

sparrows (Zink and Dittman 1993, Fry and Zink 1998), consistent with the very short branch lengths in the likelihood tree (Fig. 3.3).

Glaciation and the effects of isolation in refugia are also evident; for several populations have divergence estimates that predate the last glacial maximum (>10,000 ybp; Hamilton et al. 1986). Much of the current range of song sparrows in Alaska is thought to have been completely glaciated during the last glacial maximum (Hamilton et al. 1986). Thus, a very recent expansion into the entire Alaska range was likely. However, coalescence estimates of divergence dates suggest that there were three or four refugial populations within the current distribution of western song sparrows that have divergence estimates > 10,000 ybp. These include several previously identified refugial areas in southeast Alaska and the Queen Charlotte Islands (Scudder and Gessler 1989, Byun et al. 1997) and an only recently identified location in the Aleutian Islands (Holder et al. 1999, Congdon et al. 2000). The occurrence of refugia within the peripheral distribution of this species shows the complexity that historical factors can add to what had appeared to be a simple system of sequentially peripheral populations.

Gene flow and migration

The ability to connect a classic model of gene flow with a system that mirrors many of the facets of that model is an ideal rarely achievable in the natural world. Because of their distribution, song sparrows in Alaska appear to be a perfect case in which classic stepping-stone versus island models can be examined. Both historical gene flow estimates (Table 3.3) as well as the phylogenetic and distance based trees (Figs. 3,5) indicate that in most cases song sparrows followed a stepping-stone model of gene flow

during colonization. This might be an expected conclusion given the linear distribution of these peripheral populations, but its demonstration is important in a volant species. However, there appears to be very little if any current gene flow among song sparrow populations, regardless of their geographic proximity (Table 3.4, 3.5). Thus, while historically song sparrows often exchanged genes with neighboring populations, probably in connection with the founding of these populations, current gene flow is not sufficient to counteract genetic divergence (Hartl and Clark 1997).

Refreshment of genetic diversity in peripheral isolates nearer to the species' main range is more likely given the migratory behavior of more centrally-located populations of song sparrows. This idea is supported by the historically high levels of gene flow among migratory populations found in southeast Alaska and neighboring areas (Table 3.3). However, high historical gene flow (the highest estimate is 3.91 individuals per generation) is found between two non-migratory populations within the subspecies *maxima*. Thus, current sedentary populations do not reflect past movement capabilities. This pattern shows the continued ability to colonize new areas after populations were isolated in glacial refugia where migratory behavior was probably not present. Inferences can be made about the timing of when sedentary behavior became prevalent in Alaska song sparrows based on the estimated dates of divergence between non-migratory Alaska Peninsula populations and migratory Kodiak Island and Copper River Delta populations. Divergence estimates between these populations is approximately 6,600 - 9,300 ybp, which suggests that sedentary behavior developed in a fairly short period of evolutionary time. However, Berthold (1988) was able to alter the behavior of partially migratory

birds to being fully migratory or fully sedentary with as little as five generations of directional artificial selection. In addition, previous modeling studies of how traits are affected by peripheral isolation found that when gene flow is interrupted populations can evolve rapidly to new states (Garcia-Ramos and Kirkpatrick 1997). Thus, while song sparrows appear to be a case in which increasing peripheral isolation and loss of gene flow in extreme habitats can lead to rapid behavioral changes, the rate of loss of migratory behavior based on population divergences can only be taken as a rough estimate. However, this estimate can be useful in understanding the process of subspecific divergence or the first steps on the road to speciation.

Genetic diversity and effective population sizes

Genetic diversity declines precipitously as populations are established farther away from the species' continental distribution (Fig. 3.4). However, recent instances of bottlenecks are not apparent. Estimates based on mtDNA suggest that those populations that currently show low diversity values (e.g. Attu and Adak islands, Alaska Peninsula, and Kodiak Island; Fig. 3.4) also had lower θ values and thus lower effective population sizes than other genetically diverse populations (given a constant mutation rate). This implies that a pattern of historically low population sizes in these areas has left an indelible imprint on the diversity of these populations. However, the effective sizes for all populations are currently similar (Table 3.5) and low. These two seemingly contradictory results suggest that at one time populations in the Aleutian Islands and nearby areas had much smaller population sizes that have since grown to the long-term effective sizes of other western song sparrow populations. This conclusion seems likely

given that southeast Alaska song sparrows do not have reduced genetic diversity or bottleneck signatures as might be expected if these populations had recently undergone a decline in size. While it is difficult to differentiate between the causes of lower genetic diversity in the most peripheral populations (e.g. long-term low population size in glacial refugia or post-glacial founder events), it seems likely that these populations have persisted for many thousands of years with effective population sizes that number only in the hundreds (Table 3.5).

Morphology

Molecular estimates of population coalescence times can be used as a yardstick to examine the timing of morphological evolution in peripheral populations. Recognized subspecific differentiation in birds can serve as a reference to morphological divergence. Many subspecies of western song sparrows are not readily identifiable using mtDNA sequences alone. However, populations that share the same morphological features readily group together in the microsatellite-based distance tree, and those recognized as morphologically distinct are genetically separate (Fig. 3.5). This indicates that morphological evolution among these populations is intermediate between the neutral evolutionary rates of mtDNA and microsatellites.

A previous study of the effects of selection on beak and body size in song sparrows showed that increased body size and beak length have inferred greater fitness on overwintering populations (Schluter and Smith 1986). Because most of the traits associated with larger size are thought to be heritable in song sparrows (Smith and Zach 1979), a logical conclusion is that the large body size and beak length found in Aleutian

Island populations is due to the increased fitness that these traits confer on individuals found in the extreme climates in which they overwinter. This idea is supported by the observation that many other populations have increased body sizes on islands (Grant 1998) and at high latitudes (e.g. Bergmann's rule; Futuyma 1998). Because the most peripheral populations of song sparrows had historically small effective population sizes, currently have limited gene flow, and are found in habitats that are marginal, the combined processes of divergent selection and genetic drift were likely causes of the morphological differentiation found today.

ACKNOWLEDGMENTS

This project was supported by the Center for Global Change and Arctic System Research, the University of Alaska Museum, the National Geographic Society, the U.S. Department of Agriculture, the National Science Foundation, and an anonymous donor. Field and technical support were provided by the U.S. Fish and Wildlife Service, U.S. Coast Guard, and U.S. Air Force. I thank the personnel at the Kodiak, Izembek, and Alaska Maritime National Wildlife Refuges for allowing use of vehicles and facilities. I also thank K. Winker, D. D. Gibson, G. M. Spellman, J. J. Weicker, R. W. Dickerman, M. C. Thompson, T. M. Boucher, D. A. Rocque, A. B. Johnson, K. G. McCracken, and T. M. Braile for technical assistance and field sampling. For helpful comments and discussion, I thank A. Denton, T. Hahn, K. Schwaegerle, and K. Winker.

REFERENCES

A.O.U. 1957. Check-list of North American birds, 5th ed. American Ornithologists' Union, Baltimore, Maryland.

Aldrich, J. W. 1984. Ecogeographical variation in size and proportions of song sparrows (*Melospiza melodia*). Ornithological Monographs No. 35, American Ornithologists' Union, Washington, D.C.

Beerli, P., and J. Felsenstein. 1999. Maximum-likelihood estimation of migration rates and effective population numbers in two populations using a coalescent approach. Genetics 152:763-773.

Berthold, P. 1988. Evolutionary aspects of migratory behavior in European warblers. Journal of Evolutionary Biology 1:195-209.

Byun, S. A., B. F. Koop, and T. E. Reimchen. 1997. North American black bear mtDNA phylogeography: implications for morphology and the Haida Gwaii glacial refugium controversy. Evolution 51:1647-1653.

Cohan, F. M., and A. A. Hoffman. 1986. Genetic divergence under uniform selection. II. Different responses to selection for knockdown resistance to ethanol among *Drosophila melanogaster* populations and their replicate lines. Genetics 114:145-163.

Congdon, B. C., J. F. Piatt, K. Martin, and V. L. Friesen. 2000. Mechanisms of population differentiation in marbled murrelets: Historical versus contemporary processes. Evolution 54:974-986.

Cornuet, J. M., and G. Luikart. 1996. Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. Genetics 144:2002-2014.

Cornuet, J. M., S. Piry, G. Luikart, A. Estoup, and M. Solignac. 1999. New methods employing multilocus genotypes to select or exclude populations as origins of individuals. Genetics 153:1989-2000.

Cox, C. B., and P. D. Moore. 1993. Biogeography: An ecological and evolutionary approach. Blackwell Science Ltd., Oxford, U.K.

Emerson, B. C. 2002. Evolution on oceanic islands: Molecular phylogenetic approaches to understanding pattern and process. Molecular Ecology 11:951-966.

Fleischer, R. C., C. E. McIntosh, and C. L. Tarr. 1998. Evolution on a volcanic conveyor belt: Using phylogeographic reconstructions and K-Ar-based ages of the Hawaiian Islands to estimate molecular evolutionary rates. Molecular Ecology 7:533-545.

Fry, A. J., and R. M. Zink. 1998. Geographic analysis of nucleotide diversity and song sparrow (Aves: Emberizidae) population history. Molecular Ecology 7:1303-1313.

Futuyma, D. J. 1998. Evolutionary Biology. Sinauer, Sunderland, Massachusetts.

Garcia-Ramos, G., and M. Kirkpatrick. 1997. Genetic models of adaptation and gene flow in peripheral populations. Evolution 51:21-28.

Gibson, D. D., and B. Kessel. 1997. Inventory of the species and subspecies of Alaska birds. Western Birds 28:45-95.

Glenn, T. 1997. Genetic bottlenecks in long-lived vertebrates: Mitochondrial and microsatellite DNA variation in American alligators and whooping cranes. Ph. D. dissertation, University of Maryland, College Park.

Grant, P. R. 1998. Patterns on islands and microevolution. Pp. 1-17 *in* P. R. Grant, ed. Evolution on islands. Oxford University Press, Oxford, U.K.

Griswold, C. K., and A. J. Baker. 2002. Time to the most recent common ancestor and divergence times of populations of common chaffinches (*Fringilla coelebs*) in Europe and North Africa: Insights into Pleistocene refugia and current levels of migration. Evolution 56:143-153.

Hackett, S. J. 1996. Molecular phylogenetics and biogeography of tanagers in the genus *Ramphocelus* (Aves). Molecular Phylogenetics and Evolution 5:368-382.

Hamilton, T. D., K. M. Reed, and R. M. Thorson. 1986. Glaciation in Alaska. Alaska Geological Society, Anchorage, Alaska.

Hancock, J. M. 1999. Microsatellites and other simple sequences: genomic context and mutational mechanisms. Pp. 1-9 *in* D. B. Goldstein and C. Schlotterer, eds. Microsatellites: Evolution and applications. Oxford University Press, Oxford, U.K.

Hanotte, O., C. Zanon, A. Pugh, C. Greig, A. Dixon, and T. Burke. 1994. Isolation and characterization of microsatellite loci in a passerine bird: The reed bunting *Emberiza schoeniclus*. Molecular Ecology 3:529-530.

Hare, M. P., and G. F. Shields. 1992. Mitochondrial-DNA variation in the polytypic Alaskan song sparrow. Auk 109:126-132.

Harshman, J. 1996. Phylogeny, evolutionary rates, and ducks. Ph. D. dissertation, University of Chicago, Chicago, Illinois.

Hartl, D. L., and A. G. Clark. 1997. Principles of population genetics, 3rd ed. Sinauer, Sunderland, Massachusetts.

Hasegawa, M. H., H. Kishino, and T. Yano. 1985. Dating of the human-ape splitting by a molecular clock of mitochondrial DNA. Journal of Molecular Evolution 21:160-174.

Holder, K., R. Montgomerie, and V. L. Friesen. 1999. A test of the glacial refugium hypothesis using patterns of Mitochondrial and Nuclear DNA sequence variation in rock ptarmigan (*Lagopus mutus*). Evolution 53:1936-1950.

Huelsenbeck, J. P., and F. Ronquist. 2001. MR-BAYES: Bayesian inference of phylogeny. Bioinformatics 17:754-755.

James, F. C. 1983. Environmental component of morphological differences in birds. Science 221:184-187.

Jeffery, K. J., L. F. Keller, P. Arcese, and M. W. Bruford. 2001. The development of microsatellite loci in the song sparrow, *Melospiza melodia*, and genotyping errors associated with good quality DNA. Molecular Ecology Notes 1:11-13.

Keller, L. F., K. J. Jeffery, P. Arcese, M. A. Beaumont, W. M. Hochachka, J. N. M. Smith, and M. W. Bruford. 2001. Immigration and the ephemerality of a natural population bottleneck: evidence from molecular markers. Proceedings of Royal Society of London, Series B 268: 1387-1394.

Klicka, J., and R. M. Zink. 1997. The importance of recent ice ages in speciation: A failed paradigm. Science 277:1666-1669.

Kornegay, J. R., T. D. Kocher, L. A. Williams, and A. C. Wilson. 1993. Pathways of lysozyme evolution inferred from the sequences of cytochrome b in birds. Journal of Molecular Evolution 37:367-379.

Larget, B., and D. L. Simon. 1999. Markov chain Monte Carlo algorithms for the Bayesian analysis of phylogenetic trees. Molecular Biology and Evolution 16:750-759.

Lesica, P., and F. W. Allendorf. 1995. When are peripheral populations valuable for conservation? Trends in Ecology and Evolution 9:753-760.

Lewis, P. O., and Zaykin, D. 2001. Genetic Data Analysis: Computer program for the analysis of allelic data. Version 1.0 (d16c). Free program distributed by the authors over the internet from http://lewis.eeb.uconn.edu/lewishome/software.html

Mayr, E. 1963. Animal species and evolution. Harvard University Press, Cambridge Massachusetts.

Mayr, E. 1982. The growth of biological thought. Harvard University Press, Cambridge Massachusetts.

McDonald, D. B., and W. K. Potts. 1997. DNA microsatellites as genetic markers at several scales. Pp. 29-49 *in* D. P. Mindell, ed. Avian molecular evolution and systematics. Academic Press, San Diego, California.

Murie, O. J. 1959. Fauna of the Aleutian Islands and Alaska Peninsula. North American Fauna No. 61, U.S. Fish and Wildlife Service, Washington D.C.

Nei, M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 89:583-590.

Nei, M., T. Maruyama, and R. Chakraborty. 1975. The bottleneck effect and genetic variability in populations. Evolution 29:1-10.

Nielsen, R., and J. Wakeley. 2001. Distinguishing migration from isolation: A Markov chain Monte Carlo approach. Genetics 158:885-896.

Paynter, R. A. 1970. Check-list of birds of the world. Vol. XIII. Museum of Comparative Zoology, Cambridge, Massachusetts.

Petren, K. 1998. Microsatellite primers from *Geospiza fortis* and cross species amplification in Darwin's finches. Molecular Ecology 7:1782-1784.

Pielou, E. C. 1991. After the ice age. University of Chicago Press, Illinois.

Piry, S., G. Luikart, and J. M. Cornuet. 1999. BOTTLENECK: A program for detecting recent effective population size reductions from allele data frequencies. Journal of Heredity 909:502-503.

Posada, D., and K. A. Crandall. 1998. Modeltest: Testing the model of DNA substitution. Bioinformatics 14:817-818.

Rannala, B., and Z. Yang. 1996. Probability distribution of molecular evolutionary trees: A new method of phylogenetic inference. Journal of Molecular Evolution 43:304-311.

Rising, J. D. 1996. A guide to the identification and natural history of the sparrows of the United States and Canada. Academic Press, San Diego, California.

Schluter, D., and J. N. M. Smith. 1986. Natural selection on beak and body size in the song sparrow. Evolution 40:221-231.

Scudder, G. G. E., and N. Gessler. 1989. The outer shores. Queen Charlotte Islands, Museum Press, Skidegate, British Columbia, Canada.

Shields, G. F., and A. C. Wilson. 1987. Calibration of Mitochondrial DNA evolution in geese. Journal of Molecular Evolution 24:212-217.

Smith, J. N. M., and R. Zach. 1979. Heritability of some morphological characters in a song sparrow population. Evolution 33:460-467.

Swofford, D. L. 2001. Phylogenetic analysis using parsimony (and other methods), ver. 4.0b10. Sinauer Associates, Sunderland, Massachusetts.

Swofford, D. L., and Selander, R. B. 1981. BIOSYS-1: A FORTRAN program for the comprehensive analysis of electrophoretic data in population genetics and systematics. Journal of Heredity 72:281-283.

Zink, R. M., and D. L. Dittman. 1993. Gene flow, refugia, and evolution of geographic variation in the song sparrow (*Melospiza melodia*). Evolution 47:717-729.

Zink, R. M., and R. C. Blackwell. 1996. Patterns of allozyme, mitochondrial DNA, and morphometric variation in four sparrow genera. Auk 113:59-67.

Table 3.1. Sampling location, subspecies, number of individuals sequenced, number of individuals genotyped, and Genbank accessions for song sparrows (*Melospiza melodia*) used in this study. Museum voucher numbers are provided on Genbank.

Location	Subspecies	Sequenced *N*	Genotyped *N*	Genbank accession
Attu Is., Aleutian Is., Alaska[a]	*maxima*	10	30	AY156386-395
Adak Is., Aleutian Is., Alaska	*maxima*	10	30	AY156396-405
Alaska Peninsula, Alaska[b]	*sanaka*	10	21	AY156406-411, 162-165
Kodiak Is., Alaska	*insignis*	4	22	AY156166-169
Copper River Delta, Alaska	*kenaiensis*	10	30	AY156412-421
Alexander Archipelago, Alaska[c]	*rufina*	4	30	AY156174-187
Hyder, Alaska	*inexspectata*	5	18	AY156161, 422-425
Queen Charlotte Is., British Columbia	*rufina*	4	24	AY156170-173
Nantucket, Massachusetts	*melodia*	2	3	AY156179-180

[a] Includes individuals from Attu Island (27) and Shemya Island (3).
[b] Includes individuals from King Cove (10), Shumagin Islands (9), and Unalaska Island (2).
[c] Includes individuals from Prince of Wales Island (17), Gravina Island (8), Revillagigedo Island (2), Heceta Island (2), and Warren Island (1).

Table 3.2. Mdiv mtDNA-based estimates of θ ($2 N_f \mu$), above diagonal, and $N_f m$, below diagonal, of song sparrow population pairs. Values in parentheses are 95% credibility intervals. See Fig. 2 for population locations.

	Attu	Adak	Alaska Pen.	Kodiak	Copper R. Delta	Hyder	Alexander A.	QCI
Attu Is.	----	0.64 (0.07-4.20)	1.01 (0.14-5.88)	1.53 (0.16-7.53)	1.80 (0.02-9.3)	2.48 (0.53-10.1)	2.33 (0.5-9.23)	1.58 (0.26-7.55)
Adak Is.	3.91 (0.23-5.00)	----	0.26 (0.01-1.68)	0.38 (0.03-2.81)	0.75 (0.08-5.06)	1.11 (0.20-5.50)	1.13 (0.24-4.61)	0.41 (0.03-2.79)
Alaska Pen.	0.09 (0.01-5.00)	0.04 (0.01-4.99)	----	0.67 (0.09-3.75)	1.09 (0.15-5.05)	1.64 (0.3-6.46)	1.48 (0.30-5.34)	0.67 (0.09-3.72)
Kodiak Is.	0.04 (0.01-5.00)	0.01 (0.01-5.00)	0.30 (0.01-4.99)	----	1.33 (0.18-6.60)	2.06 (0.39-7.73)	2.08 (0.29-6.62)	0.62 (0.08-3.46)
Copper R. Delta	0.03 (0.01-4.99)	0.01 (0.01-4.99)	0.64 (0.23-5.00)	0.58 (0.06-4.99)	----	2.40 (0.46-9.23)	2.28 (0.38-8.30)	1.34 (0.22-6.55)
Hyder	0.05 (0.01-4.97)	0.04 (0.01-5.00)	0.56 (0.10-5.00)	0.49 (0.20-5.00)	1.50 (0.26-5.00)	----	3.51 (0.80-9.54)	2.09 (0.42-7.73)
Alexander A.	0.05 (0.01-5.00)	0.04 (0.01-5.00)	0.47 (0.09-5.00)	1.36 (0.22-5.00)	1.58 (0.20-5.00)	3.71 (0.26-5.00)	----	1.96 (0.36-6.62)
Queen Charlotte	0.06 (0.01-5.00)	0.01 (0.01-5.00)	0.26 (0.10-5.00)	4.45 (0.20-5.00)	0.65 (0.26-5.00)	2.23 (0.25-5.00)	3.77 (0.25-5.00)	----

Table 3.3. Mdiv mtDNA-based estimates (corrected for unequal N_e by multiplying by θ) of T ($t/2N_e$) above diagonal, and estimated time of population divergence below diagonal, given in years before present (ybp) assuming a generation time of one year for song sparrows. Values in parentheses are 95% credibility intervals. See Fig. 2 for population locations.

	Attu	Adak	Alaska Pen.	Kodiak	Copper R. Delta	Hyder	Alexander A.	Queen Charlotte
Attu Is.	----	0.13 (0.29-6.4)	0.91 (0.40-10.1)	1.84 (0.61-15.3)	2.59 (0.97-18.0)	3.03 (1.19-24.8)	2.70 (1.12-23.3)	1.64 (0.66-15.8)
Adak Is.	5,818	----	0.67 (0.14-2.6)	1.12 (0.20-3.8)	1.32 (0.42-7.50)	1.64 (0.53-11.1)	1.22 (0.50-11.3)	1.00 (0.21-4.10)
Alaska Pen.	39,351	29,039	----	0.21 (0.05-6.69)	0.15 (0.02-10.9)	0.82 (0.13-16.4)	0.74 (0.12-14.8)	0.27 (0.07-6.70)
Kodiak Is.	79,481	48,692	9,281	----	0.53 (0.16-13.3)	1.15 (0.04-20.6)	0.92 (0.04-20.8)	0.01 (0.01-6.2)
Copper R. Delta	122,208	57,143	6,606	23,030	----	0.58 (0.10-24.0)	0.59 (0.09-22.8)	1.66 (0.19-13.4)
Hyder	130,978	71,117	35,498	49,939	24,935	----	1.05 (0.04-19.6)	17.05 (0.13-20.9)
Alexander A.	117,004	52,831	32,035	39,619	25,662	45,584	----	1.65 (0.04-19.6)
Queen Charlotte	71,134	43,307	11,602	537	71,931	738,286	71,273	----

Table 3.4. Pairwise estimates of directional gene flow (N_em) using eight song sparrow microsatellite loci. Populations listed horizontally are receiving migrants; populations providing migrants are listed vertically. See Fig. 2 for population locations.

	Attu	Adak	Alaska Pen.	Kodiak	Copper R. Delta	Hyder	Alexander A.	Queen Charlotte
Attu Is.	----	0.15	0.25	0.22	0.31	0.21	0.20	0.14
Adak Is.	0.15	----	0.12	0.14	0.19	0.21	0.22	0.19
Alaska Pen.	0.21	0.10	----	0.11	0.31	0.06	0.21	0.27
Kodiak Is.	0.20	0.27	0.04	----	0.40	0.19	0.42	0.23
Copper R. Delta	0.07	0.13	0.17	0.12	----	0.11	0.24	0.08
Hyder	0.11	0.11	0.30	0.25	0.22	----	0.42	0.07
Alexander A.	0.08	0.19	0.21	0.11	0.28	0.21	----	0.15
Queen Charlotte	0.15	0.18	0.25	0.04	0.34	0.22	0.31	----

Table 3.5. Migrate microsatellite-based estimates of θ ($4Ne\mu$) and effective populations sizes (Ne) for eight western song sparrow populations.

Population	θ	Ne
Attu Island	0.772	193
Adak Island	0.699	175
Alaska Peninsula	0.710	177
Kodiak Island	0.741	185
Copper River Delta	0.667	167
Alexander Archipelago	0.703	174
Hyder	0.700	175
Queen Charlotte Islands	0.833	208

Table 3.6. GeneClass assignment test results comparing individual microsatellite genotypes with their population of origin for nine song sparrow populations, showing mean Bayesian probability of membership in that population, number of genotypes statistically not from that population, and the number of individuals not assigned to any of the sampled populations.

Population	*N*	Mean Bayesian probability	Genotypes not from population	Genotypes not from any population
Attu Island	30	0.58 ± 0.32	0	0
Adak Island	30	0.50 ± 0.34	2	1
Alaska Peninsula	21	0.43 ± 0.34	2	1
Kodiak Island	22	0.39 ± 0.33	3	2
Copper River Delta	30	0.27 ± 0.31	2	2
Alexander Archipelago	30	0.22 ± 0.30	6	4
Hyder	18	0.16 ± 0.26	3	1
Queen Charlotte Islands	24	0.23 ± 0.24	5	1
Massachusetts	3	0.01 ± 0.01	2	2

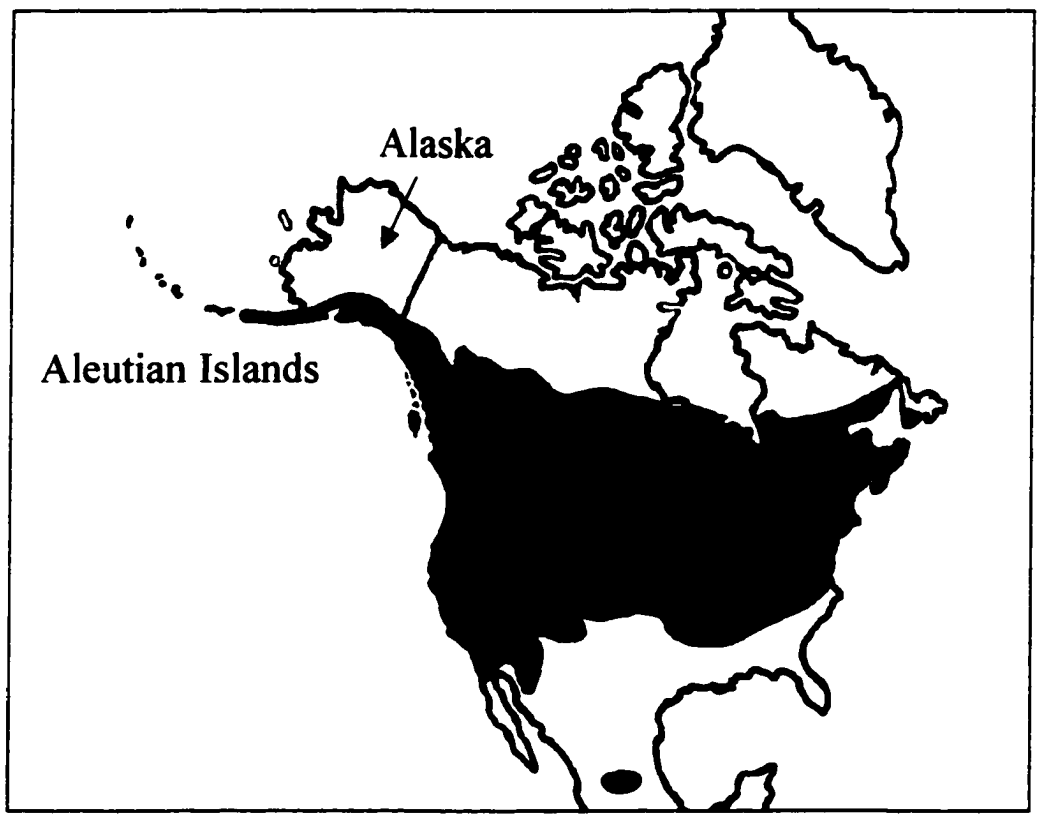

Fig. 3.1. North American breeding distribution (areas shaded in black) of song sparrows.

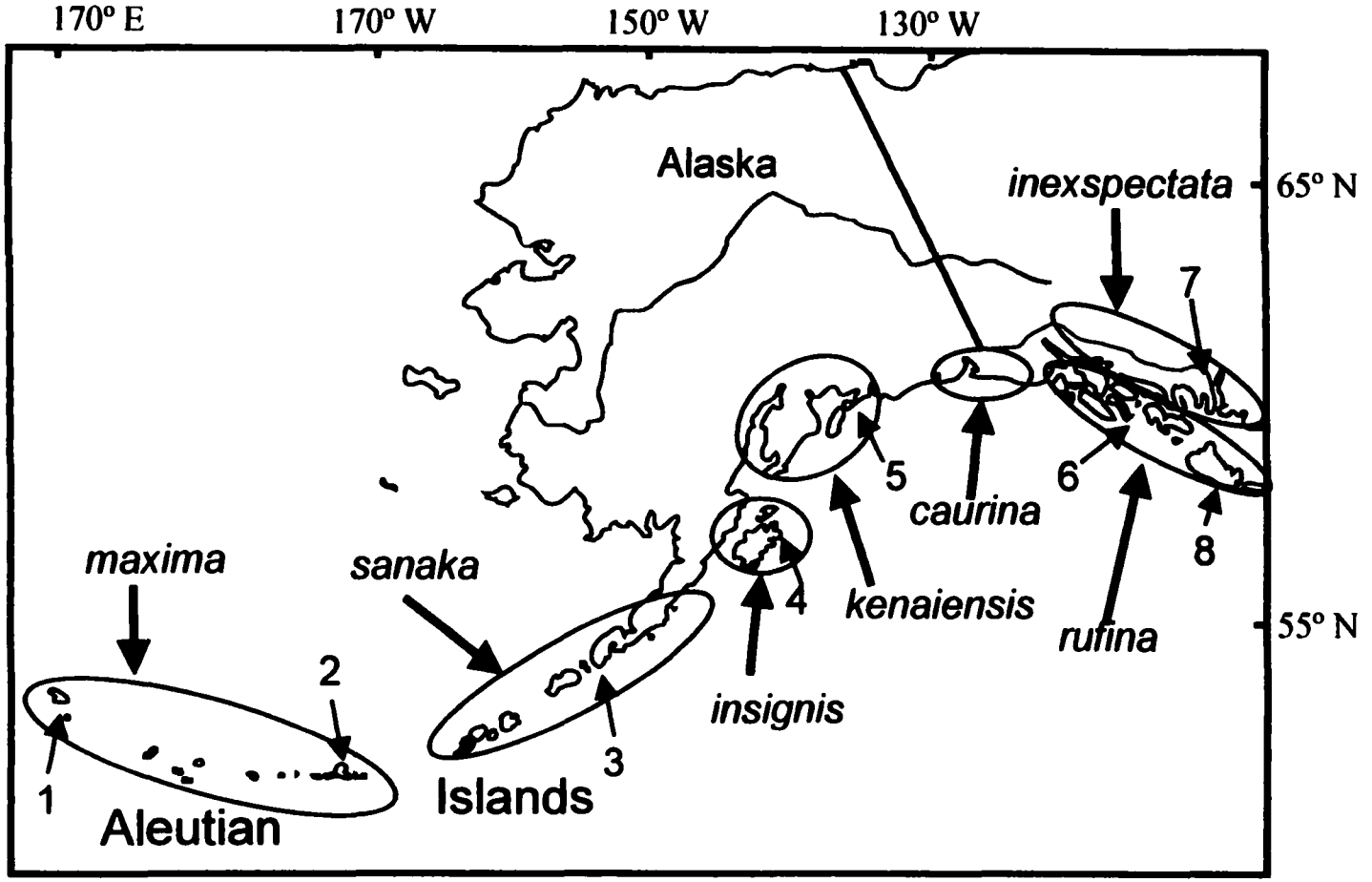

Fig. 3.2. Range of the song sparrow across the terminus of its distribution in northwestern North America. Subspecies distributions are delineated by ovals. Numbers correspond to collection locations: 1) Attu Island; 2) Adak Island; 3) Alaska Peninsula; 4) Kodiak Island; 5) Copper River Delta; 6) Alexander Archipelago; 7) Hyder; and 8) Queen Charlotte Islands.

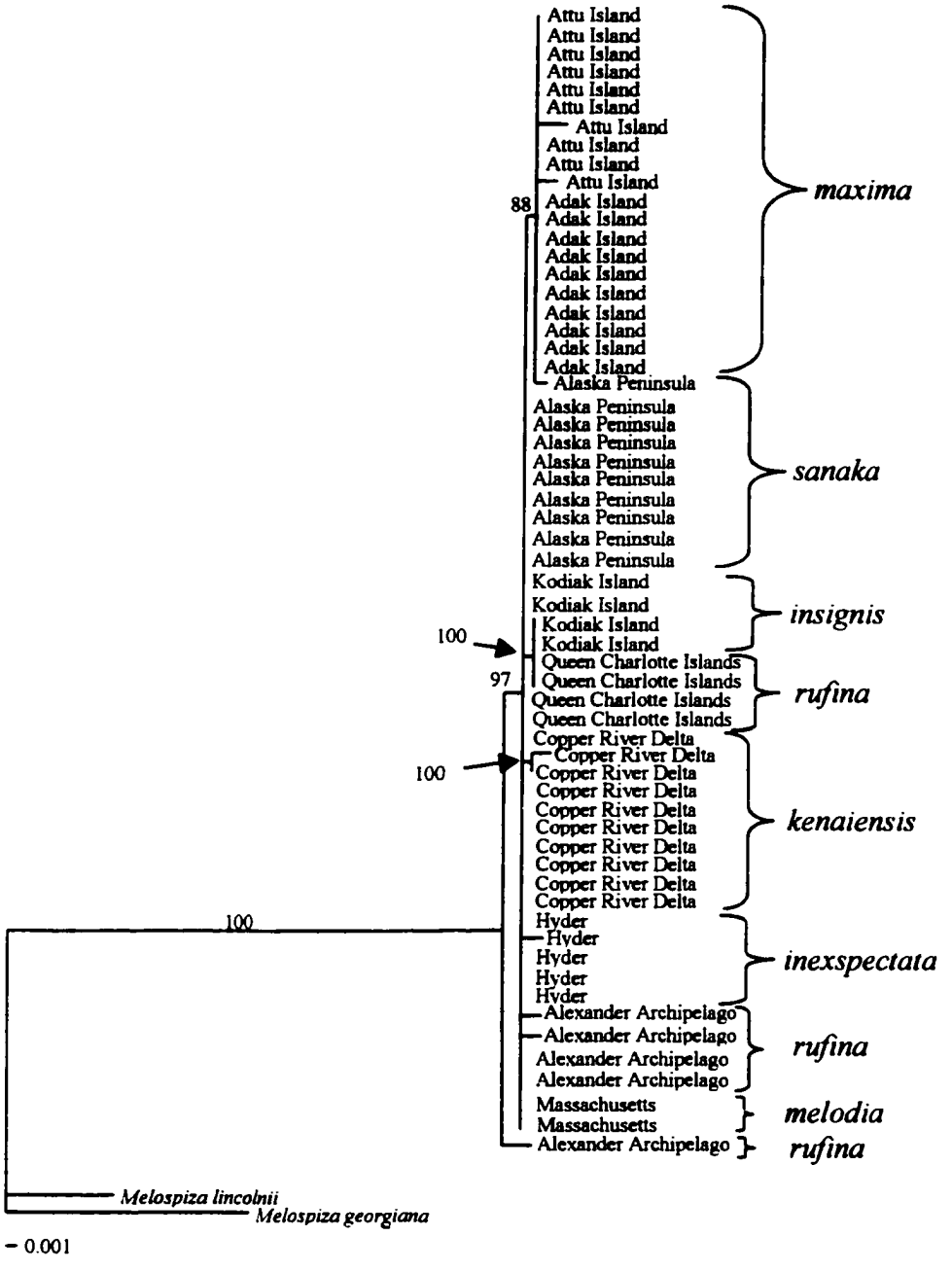

Fig. 3.3. Maximum likelihood tree of the phylogenetic relationships of Song Sparrows based on mitochondrial cytochrome b sequences and the subspecies associated with each population. The phylogram shows relative branch lengths for each individual with Bayesian posterior probabilities above each branch. Individuals with no branch lengths have identical haplotypes.

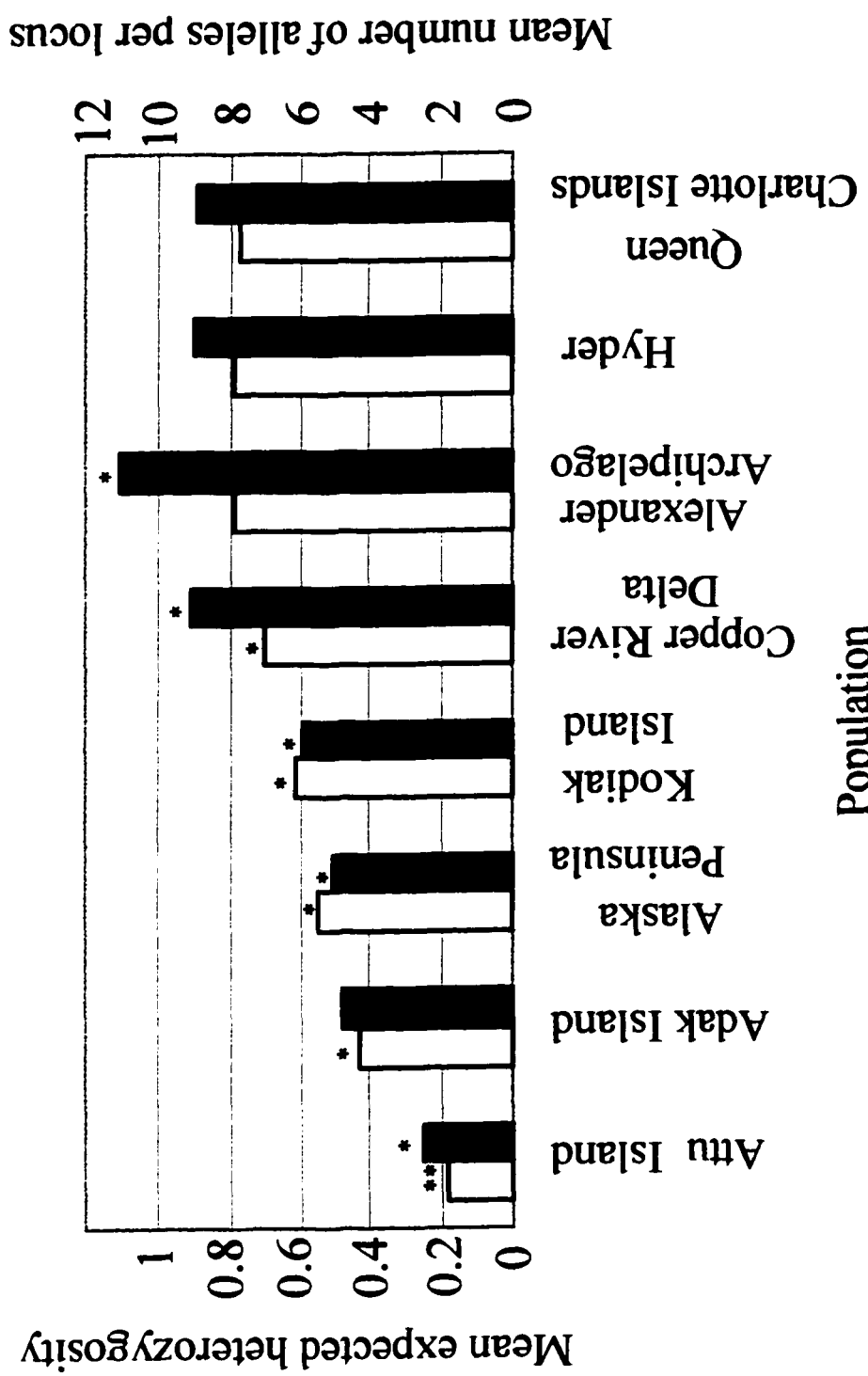

Fig. 3.4. Histogram of the mean expected heterozygosity (white bars) and mean number of alleles per locus (black bars) for each song sparrow population. Significant P-values for paired t-tests are indicated by either one ($P < 0.01$) or two ($P < 0.005$) asterisks appearing above the bar that is different from the population to the right.

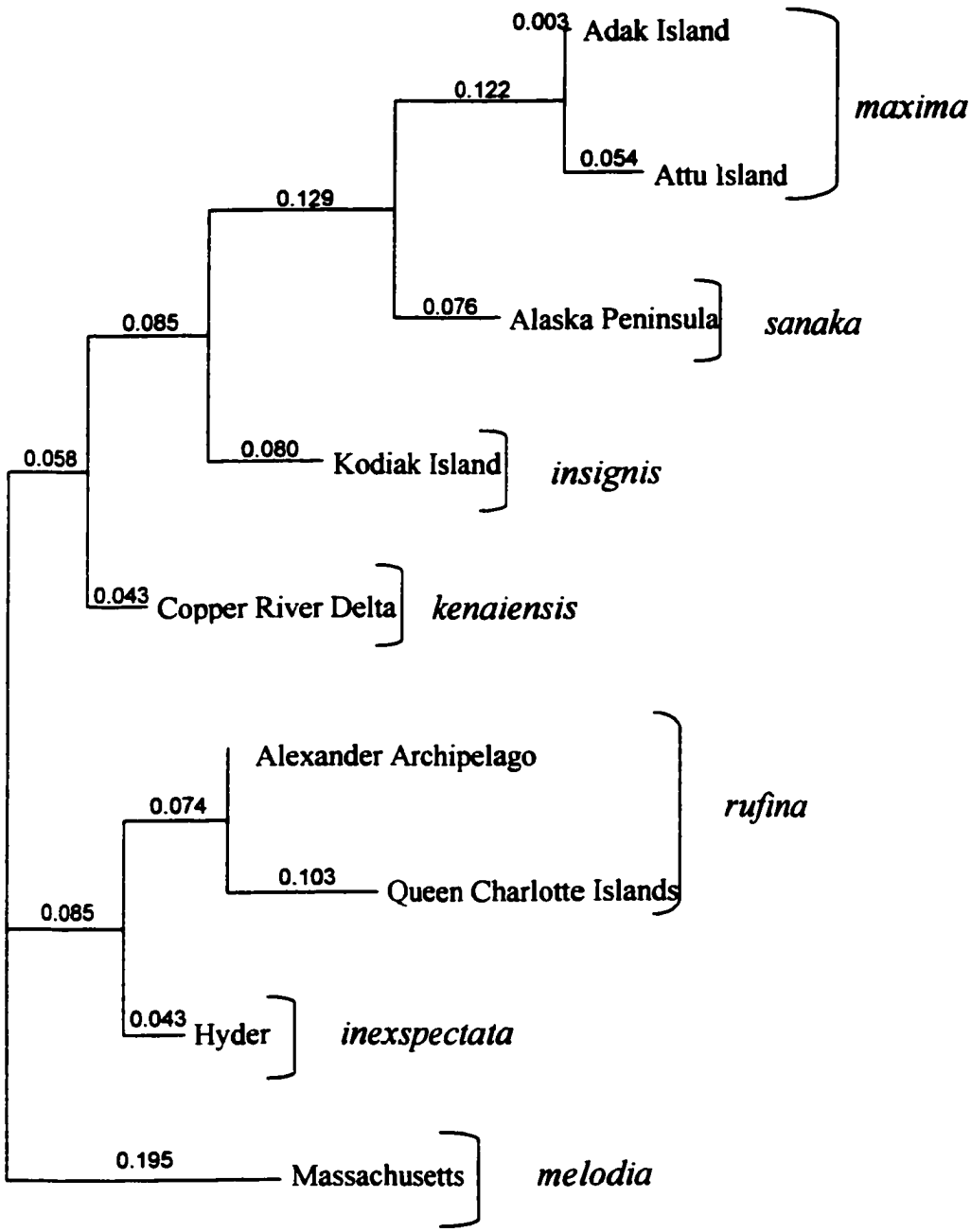

Fig. 3.5. Distance tree of song sparrow populations based on Nei's (1978) genetic distance using eight microsatellite loci. Locations corresponding to a named subspecies are bracketed. Genetic distance values are shown above branches.

IX. CONCLUSIONS

This research focused on using molecular genetic approaches to examine questions in community ecology, biogeography, and population genetics. MtDNA sequences were used to examine community assembly and the effects of climate change on a Beringian endemic species. Both MtDNA and microsatellite loci were used to examine how sequential peripheral isolation has affected population genetics in a naturally occurring vertebrate system.

Community assembly

The simple communities of landbirds found in the Aleutian Islands of Alaska were assembled through processes indistinguishable from a random assignment of colonization. Species-specific historic factors played a definitive role in Aleutian landbird community assembly in that identical end points have been achieved through different paths. Although communities currently have replicated membership this, is essentially an accident of the present, rather than the duplication of nonrandom processes. The seemingly impossible task of defining assembly rules even in such simple communities suggests that these rules might not exist in any community.

The effects of climate change on a Beringian endemic bird

Climate change and isolation in glacial refugia has shaped the current distribution and geographic variation of rock sandpipers (*Calidris ptilocnemis*). Multiple isolation events in glacial refugia and subsequent post-glacial expansions into ice-free habitats are evident. These findings suggest that climate change over the past 1.5 MY has strongly

affected the distribution and differentiation of rock sandpipers. This study illustrates the importance of climate change and refugia in the evolution of endemic Beringian species.

Genetic effects of sequential peripheral isolation

Both historic and current isolation have affected peripheral song sparrow (*Melospiza melodia*) populations. Genetic diversity in the most peripheral populations is significantly lower than in neighboring populations; however, recent bottlenecks are not indicated. The historically low effective sizes of these populations suggest that founder effects or long-term isolation in glacial refugia led to this loss in diversity. Morphological and behavioral differences among populations evolved at a rate that is intermediate between the neutral evolutionary rates of mtDNA and microsatellites. These findings, along with a lack of current gene flow, suggest that populations have differentiated through the combined processes of isolation, genetic drift, and strong directional selection. The results of this study suggest that the examination of molecular markers that evolve at different rates can provide insight into the processes that lead to subspecies differentiation or the initial stages of speciation.

X. REFERENCES

Aldrich, J. W. 1984. Ecogeographical variation in size and proportions of song sparrows (*Melospiza melodia*). Ornithological Monographs No. 35, American Ornithologists' Union, Washington, D.C.

American Ornithologists' Union (AOU). 1910. Check-list of North American Birds, 3rd ed. American Ornithologists' Union, New York.

American Ornithologists' Union (AOU). 1931. Check-list of North American Birds, 4th ed. American Ornithologists' Union, Lancaster, Pennsylvania.

American Ornithologists' Union (AOU). 1957. Check-list of North American Birds, 5th ed. American Ornithologists' Union, Baltimore, Maryland.

Avise, J. C. 1994. Molecular markers, natural history, and evolution. Chapman and Hall, New York.

Avise, J. C. 2000. Phylogeography: The history and formation of species. Harvard University Press, Cambridge, Massachusetts.

Barnosky, C. W., P. M. Anderson, and P. J. Bartlein. 1987. The northwestern U. S. during deglaciation: Vegetational history and paleoclimactic implications. Pp. 289-321 *in* W. F. Ruddiman and H. E. Wright, Jr. eds. North America and adjacent oceans during the last deglaciation. Geological Society of America, Boulder, Colorado.

Beerli, P., and J. Felsenstein. 1999. Maximum-likelihood estimation of migration rates and effective population numbers in two populations using a coalescent approach. Genetics 152:763-773.

Berthold, P. 1988. Evolutionary aspects of migratory behavior in European warblers. Journal of Evolutionary Biology 1:195-209.

Black, R. F. 1983. Glacial chronology of the Aleutian Islands. Pp. 5-10 *in* R. M. Thorson and T. D. Hamilton, eds. Glaciation in Alaska. Alaskan Quaternary Center, University of Alaska Museum, Occasional Paper No. 2, Fairbanks, Alaska.

Booth, B. D., and D. W. Larson. 1999. Impact of language, history, and choice of system on the study of assembly rules. Pp. 206-229 *in* E. Weiher and P. Keddy, eds. Biological assembly rules. Cambridge University Press, Cambridge, UK.

Borowik, O. A., and D. A. McLennan. 1999. Phylogenetic patterns of parental care in Calidridine sandpipers. Auk 116:1107-1117.

Byrd, G. V., D. D. Gibson, and D. L. Johnson. 1974. The birds of Adak Island, Alaska. Condor 76:288-300.

Byun, S. A., B. F. Koop, and T. E. Reimchen. 1997. North American black bear mtDNA phylogeography: implications for morphology and the Haida Gwaii glacial refugium controversy. Evolution 51:1647-1653.

Cody, M. L. 1975. Toward a theory of continental species diversities: Bird distributions over Mediterranean habitat gradients. Pp. 214-257 *in* M. L. Cody and J. M Diamond, eds. Ecology and evolution of communities. Harvard University Press, Cambridge, Massachusetts.

Cohan, F. M., and A. A. Hoffman. 1986. Genetic divergence under uniform selection. II. Different responses to selection for knockdown resistance to ethanol among

Drosophila melanogaster populations and their replicate lines. Genetics 114:145-163.

Congdon, B. C., J. F. Piatt, K. Martin, and V. L. Friesen. 2000. Mechanisms of population differentiation in marbled murrelets: Historical versus contemporary processes. Evolution 54:974-986.

Cornuet, J. M., and G. Luikart. 1996. Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. Genetics 144:2002-2014.

Cornuet, J. M., S. Piry, G. Luikart, A. Estoup, and M. Solignac. 1999. New methods employing multilocus genotypes to select or exclude populations as origins of individuals. Genetics 153:1989-2000.

Cox, C. B., and P. D. Moore. 1993. Biogeography: An ecological and evolutionary approach. Blackwell Science Ltd., Oxford, U.K.

del Hoyo., J., A. Elliott, and J. Sargatal. 1996. Handbook of the birds of the world, Vol. 3. Hoatzins to Auks. Lynx Editions, Barcelona, Spain.

Diamond, J. M. 1975. Assembly of species communities. Pp. 342-444 *in* M. L. Cody and J. M. Diamond, eds. Ecology and evolution of communities. Harvard University Press, Cambridge, Massachusetts.

Drake, J. A. 1990. Communities as assembled structures: do rules govern pattern? Trends in Ecology and Evolution 5:159-164.

Drake, J. A. 1991. Community-assembly mechanics and the structure of an experimental species ensemble. American Naturalist 137:1-26.

Edwards, S. V., and P. Beerli. 2000. Gene divergence, population divergence, and the variance in coalescence time in phylogeographic studies. Evolution 54:1839-1854.

Emerson, B. C. 2002. Evolution on oceanic islands: Molecular phylogenetic approaches to understanding pattern and process. Molecular Ecology 11:951-966.

Farris, J. S., M. Kallersjo, A. G. Kluge, and C. Bult. 1995a. Testing significance of incongruence. Cladistics 10:315-319.

Farris, J. S., M. Kallersjo, A. G. Kluge, and C. Bult. 1995b. Constructing a significance test for incongruence. Systematic Biology 44:570-572.

Fay, F. H., and T. J. Cade. 1959. An ecological analysis of the avifauna of St. Lawrence Island, Alaska. University of California Publications in Zoology 63:73-150.

Felsenstein, J. 1985. Confidence limits on phylogenies: An approach using the bootstrap. Evolution 29:783-791.

Fleischer, R. C., C. E. McIntosh, and C. L. Tarr. 1998. Evolution on a volcanic conveyor belt: using phylogeographic reconstructions and K-Ar-based ages of the Hawaiian Islands to estimate molecular evolutionary rates. Molecular Ecology 7:533-545.

Fox, B. J., and J. H. Brown. 1993. Assembly rules for functional groups in North American desert rodent communities. Oikos 67:358-370.

Fry, A. J., and R. M. Zink. 1998. Geographic analysis of nucleotide diversity and song sparrow (Aves: Emberizidae) population history. Molecular Ecology 7:1303-1313.

Futuyma, D. J. 1998. Evolutionary Biology. Sinauer, Sunderland, Massachusetts.

Garcia-Ramos, G., and M. Kirkpatrick. 1997. Genetic models of adaptation and gene flow in peripheral populations. Evolution 51:21-28.

Gibson, D. D. 1981. Migrant birds at Shemya Island, Aleutian Islands, Alaska. Condor 83:65-77.

Gibson, D. D., and B. Kessel. 1992. Seventy-four new avian taxa documented in Alaska 1976-1991. Condor 94:454-467.

Gibson, D. D., and B. Kessel. 1997. Inventory of the species and subspecies of Alaska birds. Western Birds 28:45-95.

Glenn, T. 1997. Genetic bottlenecks in long-lived vertebrates: Mitochondrial and microsatellite DNA variation in American alligators and whooping cranes. Ph. D. dissertation, University of Maryland, College Park.

Grant, P. R. 1998. Patterns on islands and microevolution. Pp. 1-17 *in* P. R. Grant, ed. Evolution on islands. Oxford University Press, Oxford, U.K.

Griswold, C. K., and A. J. Baker. 2002. Time to the most recent common ancestor and divergence times of populations of common chaffinches (*Fringilla coelebs*) in Europe and North Africa: Insights into Pleistocene refugia and current levels of migration. Evolution 56:143-153.

Hackett, S. J. 1996. Molecular phylogenetics and biogeography of tanagers in the genus *Ramphocelus* (Aves). Molecular Phylogenetics and Evolution 5:368-382.

Hamilton, T. D., K. M. Reed, and R. M. Thorsen. 1986. Glaciation in Alaska. Alaska Geological Society, Anchorage, Alaska.

Hancock, J. M. 1999. Microsatellites and other simple sequences: genomic context and mutational mechanisms. Pp. 1-9 *in* D. B. Goldstein and C. Schlotterer, eds. Microsatellites: Evolution and applications. Oxford University Press, Oxford, U.K.

Hanotte, O., C. Zanon, A. Pugh, C. Greig, A. Dixon, and T. Burke. 1994. Isolation and characterization of microsatellite loci in a passerine bird: The reed bunting *Emberiza schoeniclus*. Molecular Ecology 3:529-530.

Harding, R. M. 1996. New phylogenies: An introductory look at the coalescent. Pp. 15-22 *in* P. H. Harvey et al., eds. New uses for new phylogenies. Oxford University Press, UK.

Hare, M. P., and G. F. Shields. 1992. Mitochondrial-DNA variation in the polytypic Alaskan song sparrow. Auk 109:126-132.

Harshman, J. 1996. Phylogeny, evolutionary rates, and ducks. Ph. D. dissertation, University of Chicago, Chicago, Illinois.

Hartl, D. L., and A. G. Clark. 1997. Principles of population genetics, 3rd ed. Sinauer, Sunderland, Massachusetts.

Harvey, P. H., A. J. L. Brown, J. M. Smith, S. Nee. 1996. New uses for new phylogenies. Oxford University Press, Oxford, U. K.

Hasegawa, M. H., H. Kishino, and T. Yano. 1985. Dating of the human-ape splitting by a molecular clock of mitochondrial DNA. Journal of Molecular Evolution 21:160-174.

Hayman, P., J. Marchant, and T. Prater. 1986. Shorebirds: An identification guide to the waders of the world. Houghton Mifflin Company, Boston, Massachusetts.

Helm-Bychowski, K., and J. Cracraft. 1993. Recovering phylogenetic signal from DNA sequences: Relationships within the corvine assemblage (class Aves) as inferred from complete sequences of the mitochondrial DNA cytochrome-b gene. Molecular Biology and Evolution 10:1196-1214.

Hewitt, G. 2000. The genetic legacy of the Quaternary ice ages. Nature 405:907-913.

Hillis, D. M., C. Moritz, and B. K. Mable. 1996. Molecular systematics. Sinauer, Sunderland, Massachusetts.

Holder, K., R. Montgomerie, and V. L. Friesen. 2000. Glacial vicariance and historical biogeography of rock ptarmigan (*Lagopus mutus*) in the Bering region. Molecular Ecology 9:1265-1278.

Holder, K., and R. Montgomerie. 1993. Rock ptarmigan (*Lagopus mutus*). Pp. 1-24 *in* A. Poole and F. Gill, eds. The birds of North America, No. 51. The American Ornithologists' Union, Philadelphia, PA and Academy of Natural Sciences, Washington, D. C.

Holder, K., R. Montgomerie, and V. L. Friesen. 1999. A test of the glacial refugium hypothesis using patterns of mitochondrial and nuclear DNA sequence variation in rock ptarmigan (*Lagopus mutus*). Evolution 53: 1936-1950.

Hopkins, D. M. 1967. The Bering land bridge. Stanford University Press, Stanford, California.

Hopkins, D. M. 1967. The Cenozoic history of Beringia – A synthesis. Pp. 451-484 *in* D. M. Hopkins, ed. The Bering land bridge. Stanford University Press, Stanford, California.

Hopkins, D. M., J. V. Matthews, Jr., C. E. Schweger, and S. B. Young. 1982. Paleoecology of Beringia. Academic Press, New York.

Huelsenbeck, J. P., and F. Ronquist. 2001. MR-BAYES: Bavesian inference of phylogeny. Bioinformatics 17:754-755.

Hultén, E. 1960. Flora of the Aleutian Islands, 2nd ed. J. Cramer, New York.

James, F. C. 1983. Environmental component of morphological differences in birds. Science 221:184-187.

Jeffery, K. J., L. F. Keller, P. Arcese, and M. W. Bruford. 2001. The development of microsatellite loci in the song sparrow, *Melospiza melodia*, and genotyping errors associated with good quality DNA. Molecular Ecology Notes 1:11-13.

Keller, L. F., K. J. Jeffery, P. Arcese, M. A. Beaumont, W. M. Hochachka, J. N. M. Smith, and M. W. Bruford. 2001. Immigration and the ephemerality of a natural population bottleneck: evidence from molecular markers. Proceedings of Royal Society of London, Series B 268: 1387-1394.

Kidd, M. G., and V. L. Friesen. 1998. Analysis of mechanisms of microevolutionary change in *Cepphus* guillemots using patterns of control region variation. Evolution 52:1158-1168.

Klicka, J., and R. M. Zink. 1997. The importance of recent ice ages in speciation: a failed paradigm. Science 277: 1666-1669.

Kocher, T. D., W. K. Thomas, A. Meyer, S. V. Edwards, S. Pääbo, F. X. Villablanca, and A. C. Wilson. 1989. Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers. Proceedings of the National Academy of Science USA 86:6196-6200.

Kornegay, J. R., Kocher, T. D., Williams, L. A., and Wilson, A. C. 1993. Pathways of lysozyme evolution inferred from the sequences of cytochrome b in birds. Journal of Molecular Evolution 37:367-379.

Krajewski, C., and D. G. King. 1996. Molecular divergence and phylogeny: Rates and patterns of cytochrome b evolution in cranes. Molecular Biology and Evolution 13:21-30.

Landweber, L. F., and A. P. Dobson. 1999. Genetics and the extinction of species. Princeton University Press, Princeton, New Jersey.

Larget, B., and D. L. Simon. 1999. Markov chain Monte Carlo algorithms for the Bayesian analysis of phylogenetic trees. Molecular Biology and Evolution 16:750-759.

Lesica, P., and F. W. Allendorf. 1995. When are peripheral populations valuable for conservation? Trends in Ecology and Evolution 9:753-760.

Lewis, P. O., and Zaykin, D. 2001. Genetic Data Analysis: Computer program for the analysis of allelic data. Version 1.0 (d16c). Free program distributed by the authors over the internet from http://lewis.eeb.uconn.edu/lewishome/software.html

Losos, J. B. 1996. Phylogenetic perspectives on community ecology. Ecology 77:1344-1354.

M'Closkey, R. T. 1978. Niche separation and assembly in four species of Sonoran desert rodents. American Naturalist 112:683-694.

Mann, D. H., and D. M. Peteet. 1994. Extent and timing of the last glacial maximum in southwestern Alaska. Quaternary Research 42:136-148.

Mayr, E. 1963. Animal species and evolution. Harvard University Press, Cambridge Massachusetts.

Mayr, E. 1982. The growth of biological thought. Harvard University Press, Cambridge Massachusetts.

McDonald, D. B., and W. K. Potts. 1997. DNA microsatellites as genetic markers at several scales. Pp. 29-49 *in* D. P. Mindell, ed. Avian molecular evolution and systematics. Academic Press, San Diego, California.

Moore, W. W., and V. R. DeFilippis. 1997. The window of taxonomic resolution for phylogenies based on mitochondrial cytochrome b. Pp. 84-115 *in* D. P. Mindell, ed. Avian molecular evolution and systematics. Academic Press, San Diego, California.

Muira, G. I., and S. V. Edwards. 2001. Cryptic differentiation and geographic variation in genetic diversity of Hall's babbler *Pomastostomus halli*. Journal of Avian Biology 32:102-110.

Murie, O. J. 1959. Fauna of the Aleutian Islands and Alaska Peninsula. North American Fauna No. 61, U. S. Fish and Wildlife Service, Washington D. C.

Murphy, W.J., E. Eizirik, S. J. O'Brien, O. Madsen, M. Scally, C. J. Douady, E. Teeling, O. A. Ryder, M. J. Stanhope, W. W. de Jong, and M. S. Springer. Resolution of the early placental mammal radiation using Bayesian phylogenetics. Science 294:2348-2351.

Nei, M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 89:583-590.

Nei, M., T. Maruyama, and R. Chakraborty. 1975. The bottleneck effect and genetic variability in populations. Evolution 29:1-10.

Nielsen, R., and J. Wakeley. 2001. Distinguishing migration from isolation: A Markov chain Monte Carlo approach. Genetics 158:885-896.

Nunn, G. B., J. Cooper, P. Jouventin, C. J. R. Robertson, and G. G. Robertson. 1996. Evolutionary relationships among extant albatrosses (Procellariifromes: *Diomedeidae*) established from complete cytochrome b gene sequences. Auk 113:784-801.

Paynter, R. A. 1970. Check-list of birds of the world. Vol. XIII. Museum of Comparative Zoology, Cambridge, Massachusetts.

Peters, J. L. 1934. Check-list of the birds of the world. Harvard University Press, Cambridge, Massachusetts.

Petren, K. 1998. Microsatellite primers from *Geospiza fortis* and cross species amplification in Darwin's finches. Molecular Ecology 7:1782-1784.

Pielou, E. C. 1991. After the ice age. University of Chicago Press, Illinois.

Piry, S., G. Luikart, and J. M. Cornuet. 1999. BOTTLENECK: A program for detecting recent effective population size reductions from allele data frequencies. Journal of Heredity 909:502-503.

Posada, D., and K. A. Crandall. 1998. Modeltest: Testing the model of DNA substitution. Bioinformatics 14:817-818.

Price, T., I. J. Lovette, E. Bermingham, H. L. Gibbs, and A. D. Richman. 2000. The imprint of history on communities of North American and Asian warblers. American Naturalist 156:354-367.

Pruett, C. L, D. D. Gibson, and K. Winker. 2001. Molecular "cuckoo clock" suggests listing of western yellow-billed cuckoos may be warranted. Wilson Bulletin 113:228-231.

Rand, D. M. 1996. Neutrality tests of molecular markers and the connection between DNA polymorphism, demography, and conservation biology. Conservation Biology 10:665-671.

Randi, E. 1996. A mitochondrial cytochrome b phylogeny of the *Alectoris* partridges. Molecular Phylogenetics and Evolution 6:214-227.

Rannala, B., and Z. Yang. 1996. Probability distribution of molecular evolutionary trees: A new method of phylogenetic inference. Journal of Molecular Evolution 43:304-311.

Ricklefs, R. E. 1987. Community diversity: Relative roles of local and regional processes. Science 235:167-171.

Ricklefs, R. E., and D. Schluter. 1993. Species diversity in ecological communities. University of Chicago Press, Illinois.

Ricklefs, R. E., and E. Bermingham. 2001. Nonequilibrium diversity dynamics of the Lesser Antillean avifauna. Science 294:1522-1524.

Rising, J. D. 1996. A guide to the identification and natural history of the sparrows of the United States and Canada. Academic Press, San Diego, California.

Rogers, A. R. 1995. Genetic evidence for a Pleistocene population explosion. Evolution 49:608-615.

Schluter, D., and J. N. M. Smith. 1986. Natural selection on beak and body size in the song sparrow. Evolution 40:221-231.

Schneider, S., J. Kueffer, D. Roessli, and L. Excoffier. 1997. Arlequin ver. 1.1: A software for population genetic data analysis. Genetics and Biometry Laboratory, University of Geneva, Switzerland. http://lgb.unige.ch/arlequin/.

Scudder, G. G. E., and N. Gessler. 1989. The outer shores. Queen Charlotte Islands, Museum Press, Skidegate, British Columbia, Canada.

Shields, G. F., and A. C. Wilson. 1987. Calibration of mitochondrial DNA evolution in geese. Journal of Molecular Evolution 24:212-217.

Simberlof, D., L. Stone, and T. Dayan. 1999. Ruling out a community assembly rule: the method of favored states. Pp. 58-74 *in* E. Weiher and P. Keddy, eds. Biological assembly rules, perspectives, advances, retreats. Cambridge University Press, Cambridge, UK.

Smith, J. N. M., and R. Zach. 1979. Heritability of some morphological characters in a song sparrow population. Evolution 33:460-467.

Sorenson, M.D., J.C. Ast, D.E. Dimcheff, T. Yuri & D.P. Mindell. 1999. Primers for a PCR-based approach to mitochondrial genome sequencing in birds and other vertebrates. Molecular Phylogenetics and Evolution 12: 105-114.

Stewart, J. R., and A. M. Lister. 2001. Cryptic northern refugia and the origins of the modern biota. Trends in Ecology and Evolution 16:608-613.

Swofford, D. L. 2001. Phylogenetic analysis using parsimony (and other methods), ver. 4.0b10. Sinauer Associates, Sunderland, Massachusetts.

Swofford, D. L., and Selander, R. B. 1981. BIOSYS-1: A FORTRAN program for the comprehensive analysis of electrophoretic data in population genetics and systematics. Journal of Heredity 72:281-283.

Tajima, F. 1989. Statistical method for testing the neutral mutation hypothesis of DNA polymorphism. Genetics 123:585-595.

Weiher, E., and P. Keddy. 1999. Biological assembly rules: Perspectives, advances, retreats. Cambridge University Press, Cambridge, UK.

Weins, J. A. 1989. The ecology of bird communities. Vol 1. Foundations and patterns. Cambridge University Press, Cambridge, UK.

Williams, M., D. Dunkerley, P. De Deckker, P. Kershaw, and J. Chappell. 1998. Quaternary Environments, 2nd ed. Arnold, London, U. K.

Zhaxybayeva, O., and J. P. Gogarten. Bootstrap, Bayesian probability and maximum likelihood mapping: exploring new tools for comparative genome analyses. BMC Genomics 3: 4

Zink, R. M., and D. L. Dittman. 1993. Gene flow, refugia, and evolution of geographic variation in the song sparrow (*Melospiza melodia*). Evolution 47:717-729.

Zink, R. M., and R. C. Blackwell. 1996. Patterns of allozyme, mitochondrial DNA, and morphometric variation in four sparrow genera. Auk 113:59-67.